1885

1. Thyroid hormones
2. myelotropic hormones
3. thyroxin
4. thermolhyrins (FW)
5. thyroid gland

286.	13/1/61.	10/1/61

THE THYROID HORMONES

THE THYROID HORMONES AND THEIR ACTION

BY

G. MANSFELD, M.D.

Professor of Physiology
University of Budapest

TRANSLATED BY

ERWIN PULAY, M.D.

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PREFACE

THE knowledge that my book will be published in English is of particular significance to me because it was in England that I received my earliest inspiration when I studied there as a pupil of E. H. Starling. I was privileged to be the first guest of the new Physiological Institute of the University College, London, established in 1909, and there I worked under Starling and his distinguished collaborator, W. M. Bayliss. As a citizen of a foreign country I brought back from England not only newly acquired knowledge of Science but—since after all Science is universal—also that which I cherished far more, an acquaintance with the English spirit of research which is characterized not only by perseverence and profound penetration into problems, but above all by a spirit of freedom from dogma and the courage to deviate from long-established ways. If now, after nearly forty years, I venture to present to the English-speaking world this small book which represents the labour of a great part of my life, I feel I am serving the spirit of Starling and his contemporaries, who always endeavoured to conceive Life as a unity and strove to investigate the mechanism of the *Concensus partium*. In doing this, my first word of appreciation must be tendered to those who have selected my modest contribution on the regulative processes of the higher animals—a subject which I hope may be extended through our latest findings in this field—and who have facilitated its publication in English. First I wish to express my thanks to Messrs. Frederick Muller Ltd., the publishers, for having brought out this book in so attractive a form, and to the translator, Dr. Erwin Pulay, who has honoured me by suggesting that this English translation be made and by furthering its execution through his friendly and painstaking co-operation. But above all I am gratefully indebted to all my co-workers who were of great help to me during the work recorded here.

G. MANSFELD

Budapest,
September 1948

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INTRODUCTION

In an age when biochemical research rejoices in its greatest triumphs and when, almost daily, the nature of new hormones and vitamins is unfolded so that material knowledge of organisms has made strides beyond all expectation, it is not surprising that interest in *function and structure* of organisms should fall back and pure experimental physiology, especially research in regulations, receive niggardly treatment. At the turn of the century, facing the great achievements of *Pettenkofer, Voit* and *Rubner,* physiology had no other concern but that of metabolic processes, linked with energy, and visualized mathematical formulation of vital functions as its ultimate aim. Though we are indebted to this line of research for valuable results, it has led to inevitable disappointment, for, as *Uexküll** rightly says, with research in animal physiology completely dominated by the physical side a deplorable decrease of problems and aims was certain to ensue.

The consideration of organisms as mere lifeless machines, the collection of data of the mechanical and thermal qualities of single organs, diverted physiology from its highest aim of *research into the organic system as a whole* and the subjugation of the exploration of its elements to the end. About this Uexküll says: "Only an organism can live, and each organism consists of living organs, and these living organs of yet other living organs. As long as the whole is alive the separate parts live with each other and *through* each other according to a fixed plan."

At one time the lines of investigation of this plan were indicated by men of genius such as *Johannes Müller, Claude Bernard, Paul Bert, Brown-Sequard, Carl Ludwig, Goltz, Gaskell*; although biochemistry now enriches our knowledge so valuably with the identification of vital substances, it should not be forgotten that we owe the discovery of these substances, their nature, and their mode of action, as well as their significance for the organism as a whole, to these great masters of experimental physiology.

In this fixed plan, in which the parts of the organism serve the purpose of life, the so-called regulations play a part of great importance. As we know, the adaptation processes of living animals are expressions of the functioning of two systems of organs which, until recently, were sharply differentiated from each other. The phylogenetically younger nervous system in the service of

* I. v. Uexküll: *Manual for the study of experimental biology of aquatic animals.* Bergmann, Wiesbaden, 1905.

swift functional changes was opposed to the endocrine gland system which has to intervene with its slow messengers in the processes within the cell. The discovery of the chemical transmission of the nerve impulse by *O. Loewi* and the closely linked work of *H. H. Dale* destroyed this barrier; we recognized the same principle of action in both: the despatch of chemical substances which influence decisively the life of the cell.

The recognition that the nervous system also represents an organ of chemical reflexes, like the endocrine glands, made the so-called neuro-hormonal relations appear even more intimate. Apart from the variation of time scarcely any difference exists in the way in which both systems are effective, for there are reactions of the same nature whether a nervous stimulus produces acetylcholine leading to muscular contraction or when the interpolation of an endocrine gland, producing adrenaline, makes the breakdown of the liver glycogen possible.

In the range of action of the two systems, the nervous system with both its hormones, adrenaline and acetylcholine, has more influence on the specific functions of the organs, while the endocrine glands provide for the proper working of the cell, this being the preliminary condition for any undisturbed functioning of the organs. So far no certain evidence has been presented as to the *direct* influence of the nerve centres on metabolic processes without change of specific function. Actually, an exact analysis of the effect of thyroxin led to the result, as we shall see, that the so-called metabolic centres of the mid-brain do not affect the cells of the organ directly, but only through the intervention of the endocrine glands.

Of the endocrine glands that influence cell economy, that is, metabolic processes, the thyroid has always been to the fore. The feeding of thyroid substance or the dispensing of its active matter established the view that we are dealing with a powerful catalyst of metabolic processes. It seemed justifiable to relate all quantitive changes from basal metabolism to increased or diminished functioning of the thyroid gland. This view, which was also confirmed by the clinical symptoms of Graves' disease and of myxoedema, certainly gave a clear picture of the activity of this organ as such, but hardly anything was known of the significance of this activity for the organism as a whole and its character as regulator of the functions of life.

Insight into this regulative activity of the thyroid gland was not gained until it became apparent that it did not only intervene by its inner conditioning in the processes of metabolism, as has been supposed, but that it plays the additional role of intermediary between the external and internal world, thus being an important

factor in the processes which enable the organism to adapt itself to its environment.

Two observations which I made almost thirty years ago made the thyroid recognizable as an important link between the organism and the outer world. One of them concerned lack of oxygen which, as experiments showed, caused increased activity of the thyroid gland, whether conditioned by internal or external factors. The other observation pointed to the fact that yet another external factor, the temperature of the environment, influences thyroid activity; for it was evident that, both in cold as well as in warm surroundings, substances were furnished to the blood which change the consumption of substances in the organ cells in accordance with the chemical heat regulation.

These observations had already shown that the thyroid gland has many functions in addition to the acceleration of combustion. This led to a series of experiments which provide evidence that the thyroid gland occupies no unimportant place in the mechanism of regulations. Firstly, an exact analysis of the mechanism of activity and point of attack of thyroxin established the fact that there is a third environmental factor which exerts a decisive influence on the thyroid gland: the beginning of the warm season which, apparently irrespective of variations of temperature, causes the formation of an active substance which is absent during the winter months. These investigations were the experimental basis of the assumption that, in addition to thyroxin, the thyroid gland produces other active substances able to influence metabolic processes in the cells and so to intervene in the highly complicated process of heat regulation.

Closer investigation of the reaction of the thyroid gland to lack of oxygen mentioned earlier revealed that the gland was also capable of controlling blood formation processes and, by producing a particular hormone, of providing conditions for a normal process of blood formation in the bone-marrow.

It was necessary to subject the findings of these experiments to chemical research and the combination of these two led to the discovery of hitherto unknown active substances of the thyroid gland which, as we shall see, are significant beyond the bounds of physiology, and may prove of importance in clinical research. Whether these findings, the result of three decades of research work on the thyroid gland, will be useful in the solution of the burning questions and numerous enigmas of the pathology of the thyroid gland is yet to be seen. The assembly of these results originated in the desire for establishing a collaboration between clinical and experimental medicine, for only through this can the new field of discoveries bring benefit to the patient.

For the sake of clarity the book has not been planned as a chronological sequence of individual efforts and care has been taken to avoid filling the gaps in our knowledge with theories and comments. For nothing is more paralysing for future research, the stimulation of which is the main object of this book, than the apparent solution of problems in which medical science is most excessively rich.

PART ONE

THE "MYELOTROPIC HORMONE"

CHAPTER I

OXYGEN DEFICIENCY, THE THYROID GLAND, AND BLOOD FORMATION

THE physiology of the relationships between blood formation, and oxygen deficiency received an important advance from P. Bert's[1] discovery of the influence of sea level on the activity of blood-forming organs. Bert's researches, as well as those of Viault[2] in the Andes, Muentz[3] in the Pyrenees, and Miescher[4] in the Swiss Alps, started off numerous investigations which revealed that respiration of rarefied mountain air leads not only to a washing out of the blood corpuscles by a contraction of the spleen, as Barcroft[5] demonstrated, but also to a regeneration.

Little was known about the mechanism of this effect of oxygen deficiency on the blood-forming organs. Experiments made us presume that it was due to certain stimulating substances which enter the blood at times of a deficient oxygen supply to the organism. Carnot and Deflandre[6] and P. Th. Mueller[7] demonstrated that removal of blood and respiration of rarefied air endow the blood serum with the attribute of introducing an accelerated blood formation, which has been shown in a transference test on normal animals. These unknown, humorally transferable, serum principles were described by the French as "hemopoiétine", though nothing was known either of their origin or of their mode of action.

In 1910, I set myself the task of investigating the effect of a moderate lack of O_2 on metabolism. My attention was particularly drawn to this question by an old experience due to the experiments of Fraenkel and Bauer[8]. When animals are made to breathe gas mixtures poor in O_2, or when they are deprived of blood, or when O_2 deficiency is caused in any other way, an increased decomposition of the protein can be observed two days later. It is strange that, with thyroxin yet undiscovered and its delayed effect on metabolism unknown, I should have thought that this delayed effect of O_2 deficiency was not due to an immediate protoplasmic

response, but that the increased protein decomposition was the result of an increased activity of the thyroid gland.

Results were obtained on normal and on thyroidectomized animals (dogs and rabbits) which demonstrated clearly that lack of oxygen leads to an increase of protein metabolism only when the thyroid gland is active; otherwise it may lead to a decrease of N-elimination. (See Table I.) (Cf. G. Mansfeld and Friedr. Mueller.[9])

TABLE I

Normal				Thyroidectomized				
Experiment No.	N-elimination on the last normal day gm.	N-elimination on the second day after O_2-deficiency gm.	Difference %	Experiment No.	N-elimination on the last normal day gm.	N-elimination on the second day after O_2-deficiency gm.	Difference %	Type of O_2-deficiency
I	0·95	1·24	+30	II	1·01	0·54	—46	HCN
				III	0·99	0·63	—36	
				IV	0·72	0·67	— 6	
V	0·66	0·84	+27					
VI	0·80	1·23	+53	VII	0·54	0·48	—11	
				VIII	0·79	0·63	—20	
				IX	0·83	0·79	— 4	
X	0·85	0·97	+14	XI	0·63	0·62	—1·5	reduced air pressure
XII	1·03	1·11	+ 8	XIII	0·75	0·68	— 9	
XIV	1·43	2·29	+51					withdrawal of blood
XV	0·70	0·84	+20	XVI	0·67	0·67	0	
				XVII	0·93	0·88	— 5	

Our experiments showed that lack of oxygen most probably attacks the thyroid gland directly. By a preliminary operation on the throat of a rabbit, we cut small apertures above the carotids and kept them shut with aseptic dressing. Thus we could produce lack of O_2 in the thyroid gland and study its effect on protein metabolism without fastening up the animal or stimulating it in any other way, simply by the temporary use of clamps on the carotids.

The throttling of the blood supply for thirty minutes results two days later in an increase of nitrogen elimination, a result which would have been identical if we had exposed the whole animal to oxygen deficiency. The pinching off of the carotids on an animal without thyroid had no effect whatsoever on the N-elimination.

Hence we concluded that poorness of O_2 causes an increased activity of the thyroid gland and that the increase of protein metabolism is due to the increased activity of the thyroid.

The fact, revealed by these investigations for the first time,[10] that lack of oxygen to a moderate degree leads to increased functional activity of the thyroid gland, has, despite Hari's objections,[11] since been re-affirmed. (E. W. Baader,[12] F. Nicoletti,[13] Peisachowitsch,[14] W. Raab,[15] H. Reploh,[16] E. Schulze,[17] A. Vannotti.[18]) These authors demonstrated that respiration of minor

CO-concentrations causes an over-functioning of the thyroid gland, which leads to an increase of the basal metabolism of about 50–60 per cent, and which can be proved histologically as well as by the increased acetonitrile sensitivity of white mice. Recently Kampelmann and Schulze[19] have also proved that the stimulating effect of lack of oxygen is due to the direct thyroid influence and is not brought about via the thyreotropic hormone of the hypophysis; this is in accordance with our above-mentioned investigations.

The knowledge that lack of oxygen acts on metabolism through the medium of the thyroid gland led to the thought that the thyroid gland might also play a part in the case of increased blood formation due to oxygen deficiency, the more so as clinical observations have shown that myxoedema is associated with anaemia and that a decrease of the red blood corpuscles and haemoglobin is noticeable on thyroidectomized animals. (Dominicis,[20] Formanek and Haskovec.[21])

In 1913, I[22] reported on experiments dealing with the examination of the relationship between *oxygen deficiency, blood formation,* and *thyroid gland.* These investigations revealed two facts. Firstly, that the known effect of *high altitude* on the formation of blood takes place only on normal animals, but not on thyroidectomized ones.

From Table II it follows that our normal animals on their return from a three weeks' stay in the High Tatra (1500 m.) showed a very substantial or, at least, a marked increase of the number of blood corpuscles, whereas thyroidectomized animals revealed a decrease of the number of the blood corpuscles and of haemoglobin.

TABLE II*

Change of Blood after 20 days' stay in the Mountains.

Experiment No.	No. of Red Blood Corpuscles				Haemoglobin in 100 c.c. of Blood.		
	Initial Mill.	After 22 days Mill.	Difference Mill.	Difference %	Initial	22 days later	Difference
Normal Animals							
1	5·115	6·130	+1·015	+19·8	12·45	14·43	+19·9
2	5·110	5·930	+0·820	+16·0	15·11	14·32	— 5·2
3	5·890	6·970	+1·080	+18·0	15·09	16·10	+ 6·6
4	6·185	6·640	+0·455	+ 7·3	12·55	12·83	+ 2·2
5	5·530	6·440	+0·910	+16·4	14·66	15·25	+ 4·0
6	4·530	4·405	—0·125	— 2·7	12·00	10·83	— 9·7
Thyroidectomized Animals							
7	5·450	4·845	—0·605	—11·0	14·83	12·06	—18·0
8	4·320	4·540	+0·220	+ 5·0	12·03	10·76	—10·0
9	6·405	3·800	—2·605	—40·0	14·16	8·96	—36·0
10	4·905	4·540	—0·365	— 7·4	10·49	11·32	+ 7·8
11	5·290	4·770	—0·520	— 9·8	12·49	10·72	—13·0
12	5·510	4·280	—1·230	—22·0	12·59	11·96	— 5·0

*Mill.=millions per cubic millimetre

The second noteworthy fact was that artificial anaemia, as seen after injection of phenylhydrazine, demonstrates a much slower regeneration when the animals are deprived of their thyroid.

In Table III we can see the increase of the number of erythrocytes in 12 days in normal animals and in those without thyroid due to a toxic anaemia of the same degree.

TABLE III

Cure of Toxic Anaemia on Normal and Thyroidectomized Rabbits.

Normal Animals.					Thyroidless Animals.				
Experiment No.	Initial Mill.	After Phenyl Hydrazine injection Mill.	No. of Blood Corpuscles 12 days later Mill.	Increase %	Experiment No.	Initial Mill.	After Phenyl Hydrazine injection Mill.	No. of Blood Corpuscles 12 days later Mill.	Increase %
13	5·57	2·68	4·60	71	22	4·74	2·13	2·47	11
14	5·35	3·45	5·12	48	23	6·31	2·76	3·66	32
15	5·24	2·23	3·39	52	24	6·35	2·20	3·78	71
16	4·90	3·80	4·17	10	25	5·96	2·13	2·99	40
17	5·67	2·30	4·67	103	26	5·92	2·37	2·89	21
18	6·43	3·42	5·06	48	27	6·13	4·32	4·77	10
19	6·74	2·43	3·90	60	28	5·76	2·60	3·35	28
20	6·90	2·03	5·78	184					
21	6·72	2·05	5·66	112					
				76%					30%

Simultaneously we examined the effect of thyroid extracts on normal animals and found an appreciable increase of the blood corpuscles and of haemoglobin.

The observation of A. Ollino[23] was in harmony with these investigations. This was that extracts of the thyroid gland provoke an histologically demonstrable irritation of the bone marrow. Later investigations by Marcel Dubois,[24] Alois Waser[25] and Kiyoshi Furuya,[26] also confirmed our findings as to the role the thyroid gland plays in respect of blood formation.

The demonstrations by Eggert,[27] that the lizard *Lacerta vivipara* after removal of the thyroid dies from " aregenerative " anaemia is of great interest. Our findings are also confirmed in more recent literature. Thus, Sharpe and Bisgard[28] found, after a complete removal of the thyroid in young animals, a steady decrease of oxygen carriers and, after a supply of thyroid extract, a powerful increase of the number of erythrocytes as well as of haemoglobin. The experiments of Wadi and Loewe[29] also point in favour of the part played by the thyroid gland in the blood-forming process, because " the increase of haemoglobin and the erythrocytes—the pronounced results of an iodine treatment—does not occur in thyroidectomized animals." Also Overbeek[30] reported that " After hypophysectomy, the bone marrow appears aplastic and does not react any more to various stimuli (Pernaemon, rarefaction of the air[31]), but reacts promptly to thyroid preparations."

In order to decide whether this influence of the thyroid gland can be substituted by thyroxin, we rendered *thyroidectomized* animals anaemic by bleeding and investigated whether the rather slow regeneration in these animals could be speeded up by thyroxin. Our experiments had a negative result. *Thyroxin was completely ineffective with regard to blood formation in thyroidectomized animals.* This coincides with our later experiments, according to which, as we shall see, the part played by the thyroid in the activation of the liver principle cannot be substituted by thyroxin. Only Idia[32] saw an acceleration of the blood regeneration by thyroxin which, however, depends on the spleen and reticuloendothelium being unimpaired.

Taking all these findings into consideration, there can be no doubt that a normal activity of the thyroid gland is of importance for undisturbed blood formation and that the favourable effect of lack of oxygen in respect of the formation of blood (be it conditioned by HCN, respiration of CO or of gas mixtures poor in O_2, or through removal of blood), is mediated by the thyroid gland. We have already seen that moderate oxygen deficiency is responsible for an activation of the thyroid. The establishment of the fact that lack of oxygen does not act immediately on the bone marrow but via the thyroid gland marked, in our view, some progress in the knowledge of the physiological regulations.

As to the nature of this thyroid effect, we could ascertain, to begin with, that effective blood sera of anaemic animals with the so-called " haemopoiétines " are supplied only by animals which are in possession of their thyroid and that the thyroid is needed for their efficacy. (Mansfeld and Orban.[33]) A further clarification of this thyroid activity was made possible by our experiments of which I shall report later.

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CHAPTER II

THE RELATIONSHIP BETWEEN THE THYROID GLAND AND PERNICIOUS ANAEMIA

WHEN, some thirty years ago, we discovered the relationship between oxygen deficiency, the thyroid gland, and blood formation, which I described earlier, I considered the thyroid as a general catalyst of metabolic processes promoting the activity of the blood-forming organs, but it never occurred to me that the thyroid gland might play a role in the genesis of pernicious anaemia.

At that time, anaemia was considered to be caused by a haemolytic poison ravaging in the organism, which the bone marrow could not overcome even with its maximum activity. How, I thought, could this bone marrow, irritated to the utmost, still be influenced by the thyroid gland?

In the meantime, however, our views on this disease have undergone changes. To-day we know that it is a question not only of a quantitative, but also of a qualitative change in blood formation. We also know that the bone marrow shows a reversal of the blood formation into the embryonic paths. It is a deficiency disease of the bone marrow, the characteristic of which is that the immature cells show a slight tendency to further differentiation. In spite of hyperplasia, the bone marrow is functionally inferior and produces blood corpuscles of short life duration. That the thyroid gland might play a certain role in this unfinished process did not appear unlikely, and I found in the literature some indications which lent support to this possibility. Cases of hypothyreosis in pernicious anaemia were reported by Boros and Czoniczer,[34] and later by Vannotti.[35]

The important findings of Mendershausen[36] are very impressive. He was able to establish that in each of twelve cases who died of pernicious anaemia there was an atrophy of the *thyroid gland,* and, what is of particular interest, that atrophy could be ascertained *in this endocrine gland alone.* Mendershausen sees a possible explanation of this important pathological-anatomical discovery in the fact " that disturbances of the thyroid and the bone marrow behave in respect of one another in such a way that characteristic anaemia takes place only on the occurrence of an endocrine disorder through some new toxin. This would mean that the pre-existing disturbance of the thyroid function shows ' inclination ' towards the development of pernicious anaemia. Such an assump-

tion would find support in the experiments of Mansfeld, who produced experimental anaemia in otherwise healthy animals and observed blood regeneration take place in the sense of *hypochromic* anaemia, whereas shortly after bleeding thyroidectomized rabbits a *hyperchromic* picture appeared, which remained in existence for a long time, mostly till the *status quo ante* was reached or not quite reached. (Cf. Waelchi, Dubois.)"

As we shall see, the assumption of some sort of connection between endocrine "inclination" and an "unknown toxic agent" is in perfect harmony with the results obtained by our experiments, which were aimed at the clarification of the role of the thyroid in this process.

The discoveries of Whipple and Robscheit-Robbins,[37] and of Minot and Murphy,[38] demonstrated the importance of the liver for normal blood formation and its role in pernicious anaemia. In order to elucidate thyroid activity, we tried to find an answer to the following question: *Is this activity necessary for the formation of the anti-anaemic stimulating substance of the liver?*

For this purpose, we had to investigate whether extracts made from the liver of thyroidectomized animals were as effective as when made from the liver of normal ones. Until recently a quantitative evaluation of liver preparations was possible only on sick people. We owe it to Gottlebe[39] that to-day we are in a position to apply it to rabbits. If, daily or every second day, we give these animals injections of saponin and collargol, thereby imitating the two main symptoms of pernicious anaemia, namely haemolysis and stoppage of the reticulocytes in the bone marrow, we obtain, in two to three weeks, an anaemia of the desired degree, which after discontinuation of the above injections remains fairly constant. When we give injections of anti-anaemic preparations to rabbits previously treated in this way, the result is a crisis of the reticulocytes exactly as in pernicious anaemia plus an increase of the red blood corpuscles in proportion to the administered quantity. As not only the number of the blood corpuscles but also the duration of the effect is proportional to the injected quantity, it is best to express the strength of this effect in such a way that the change in the number of the blood corpuscles is entered in a system of co-ordinates and the enclosed surface of the curves is worked out in sq. mm.

In Diagram 1 we give an example, above, of the effect of the saponin-collargol injections, and below, of the effect of 0.3, 1.0 and 2.0 c.c. of an effective liver preparation on the same animal. This method yields good results, when a sufficiently large number of well-looked-after and well-fed animals is available. During the process of anaemizing, about 30 to 40 per cent of the animals die.

Most of the surviving ones show constant and regular anaemia, which, as soon as the liver effect is over, usually adjusts itself to the low starting value of the number of the blood corpuscles. When this is not the case and the cure is a lasting one, the experiment is of no use, because of lack of certainty that the increase was a result of the liver preparation and did not occur spontaneously.* Gottlebe's method, however, enabled us to answer the question, whether thyroid activity was necessary for the formation of an effective liver principle.

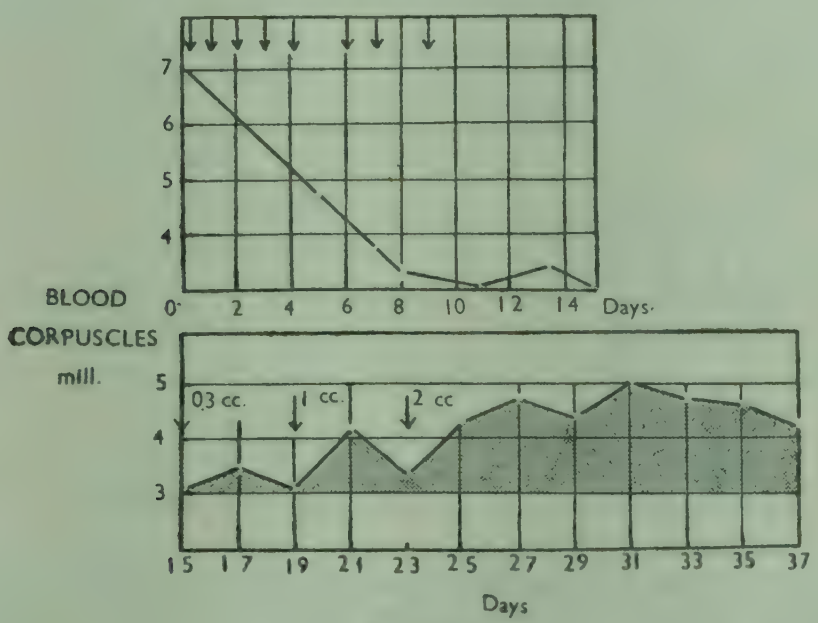


DIAGRAM 1

Above: Effect of saponin-collargol injections on a rabbit.
Below: Effect of 0.3, 1.0 and 2.0 c.c. of liver preparation on the same animal.

For this purpose, we removed the thyroid gland in ten dogs, saving each time two parathyroids. We kept these dogs alive for three months together with ten normal control animals living under the same conditions. After this period all the twenty animals were killed by bleeding. The livers of the test and control animals were then put through a mincing machine and preserved in 96% alcohol. The chemical firm, Gedeon Richter,

* Still more lasting and more regular anaemias may be achieved in thyroidectomized animals, but, as we shall see later, the liver effect there can only be obtained through a simultaneous administration of effective thyroid substance.

As regards thyroidectomized rabbits, we are about to elaborate a method with the application of an effective thyroid substance, the liver-activating effect of which seems to be to a certain extent independent of the administered quantity.

Budapest, obtained liver preparations therefrom by the usual method of Cohn's G-fraction and brought them to the same concentration.

We administered liver preparations which came from these normal and thyroidectomized dogs to five anaemized rabbits (Gottlebe's method). In these, as in all the following experiments, we worked out the surface values obtained through the administration of in all 3.0 c.c. liver preparation and displayed the average of each set of experiments in form of columns. Each column shows the average value of the curved surfaces obtained from at least five rabbits.

DIAGRAM 2

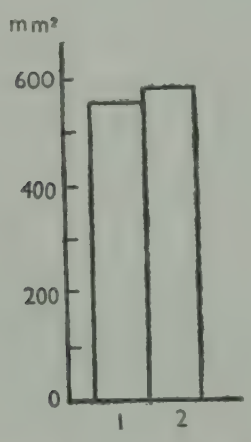


DIAGRAM 3

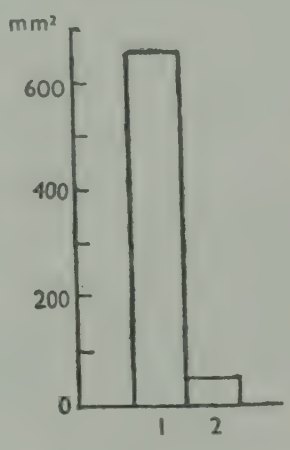


DIAGRAM 2: Effect of administration of liver preparations from (1) normal and (2) thyroidectomized dogs to anaemized rabbits.

DIAGRAM 3: Effect of the same liver preparations on normal and thyroidectomized rabbits.

In Diagram 2 we see that both liver preparations show an almost identical effect, which justifies the assumption *that activity of the thyroid gland is unnecessary for the formation of the anti-anaemic liver principle.*

After this negative result, we had to ascertain *whether the activity of the thyroid gland was needed in order to make the anti-anaemic principle of the liver effective.*

To answer this question, we applied our liver preparations to anaemized rabbits whose thyroids had been previously removed by us.

These experiments led us to a most important result. It was demonstrated *that the same preparation which was fully effective on normal rabbits failed completely on thyroidectomized ones.* (See Diagram 3.) This very interesting result indicated the direction along which might be made the discovery of the role played by the thyroid in pernicious anemia. It was demonstrated

that the anti-anaemic principle of the liver is ineffective in thyroidectomized animals and that the activity of the thyroid gland is necessary for its efficacy.

34. Boros, J., and Czoniczer, G.: *Klin. Woch.* (1935), 573.
35. Vannotti, A.: *Schweiz. med. Woch.* (1940), 1106.
36. Mendershausen, A.: *Klin. Woch.* (1925), **4**, 2105.
37. Whipple, G. H., and Robscheit-Robbins, F.S.: *J. Exp. Med.* (1933), **57**, 637, 653, 671; *Amer. J. Physiol.* (1927), 79, 260, 271; **80**, 391, 400.
38. Minot, G. R., and Murphy: *J. Amer. Med. Assoc.* (1926), **87**, 470; (1927), **89**, 759.
39. Gottlebe: *Arch. exp. Path. Pharm.* (1936), **180**, 345; **181**, 317; **182**, 91.

CHAPTER III

THE PREPARATION OF THE ACTIVE PRINCIPLE FROM THE THYROID

THE next question to arise from this knowledge was: Is this newly recognized role of the thyroid gland with regard to the activation of liver preparations associated *with the activity of the whole organ* or can it be substituted by one of its effective substances? For the examination of this question we had a magnificent subject for test in the form of an anaemized rabbit *without thyroid* (Gottlebe[39]) on which, as we have seen, the stimulating substances of the liver were ineffective. We wanted to find out whether an addition of various thyroid substances led to an activation of the liver substance. All the thyroid extracts tested below were produced in such a way that they corresponded to 1 gm. of fresh thyroid per c.c. and were thus quantitatively comparable to one another.

To begin with, we examined the possibility of substituting the missing thyroid gland by the protein-free, aqueous thyroid preparation obtained by alkaline hydrolysis, as this preparation proved to be active in the metabolic test and might contain all the effective substances of the thyroid.

As column 1 of Diagram 4 shows, this preparation proved to be effective. On the average, the effectiveness of the liver preparation on the five thyroidectomized animals treated with this "full extract" amounted to approximately 400 sq.mm., whereas on a normal animal (see Diagram 2) the effective strength of the same liver preparation was about 550 sq.mm. *Hence it follows that this activity of the thyroid gland can also be substituted by substances that can be extracted from it, and is, therefore, conditioned by hormones.*

The next question was whether it was thyroxin which was necessary for the effectiveness of the liver principle.

Column 2 of Diagram 4 shows the particularly noteworthy fact that thyroxin (Roche) was completely ineffective for the activation of the liver principle. The effect of this hormone manifested itself only in a serious crisis of the reticulocytes, without an increase of the erythrocytes, which coincides with the clinical observation that strumectomy diminishes the values of the reticulocytes; this can be rectified by administration of thyroxin.

If we treat the full extract with acid, we obtain a precipitate

which contains thyroxin and a filtrate containing acid-soluble substances of the thyroid gland.

The inefficacy of this acid-soluble fraction is shown by column 3 of Diagram 4.

When the acid precipitate containing thyroxin is brought into solution by an addition of alkali, this fraction proves to be effective, as column 4 of Diagram 4 shows. It thus became clear that the stimulating substance for which we were searching is, like thryoxin, insoluble in acid, and that it *is precipitated* during the acidification of the full extract *together with the thyroxin.*

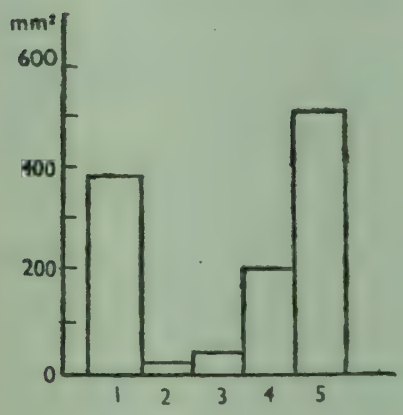


DIAGRAM 4.

Effect of liver preparations plus the undermentioned substances on anaemized thyroidectomized rabbits:

1. Protein-free aqueous thyroid preparation
2. Thyroxin (Roche)
3. Acid-soluble fraction of thyroid extract
4. Acid-insoluble fraction of thyroid extract
5. Myelotropic hormone

Our next task was the isolation of this stimulating substance, found in the acid precipitate, from thyroxin. For this purpose the so-called thyroxin fraction was boiled with 40 per cent baryta, forming a precipitate. Upon examination, the baryta-free water-soluble fraction proved almost inactive. The baryta precipitate was then disintegrated, freed from baryta, and purified through careful acidification. In this way we succeeded, as our metabolic tests demonstrated, in almost entirely removing the thyroxin from the solution and in obtaining a clear aqueous solution which, as column 5 of Diagram 4 shows, possesses full efficacy, for the thyroidectomized animals treated with this preparation regained fully their reactive faculty as regards the liver preparation. The strength here amounted to 500 sq.mm. as compared with 550 sq.mm. on a normal animal. (See column 1, Diagram 2.)

A further purification of this active preparation can be obtained through treatment with alcohol. If we mix the clear solution with a fourfold quantity of alcohol, we obtain a white precipitate which is to a large extent inorganic. If the alcoholic filtrate is evaporated *in vacuo* and brought to the original volume, a preparation showing a powerfully increased effect is obtained. The efficacy of our liver preparation rose to 900 sq.mm. on rabbits which had previously been treated with this

purified thyroid preparation; it thus was almost twice as effective as on a normal animal.

In this way we succeeded in isolating a new active substance of the thyroid gland from the thyroxin containing (acid insoluble) fraction. We called this substance *the myelotropic hormone of the thyroid gland,* assuming that its effect was directed against the bone marrow. Overbeek's[40] experiments confirmed the correctness of our assumption.

Our evidence that the activity of the thyroid gland was indispensable for the efficacy of the liver principle was also confirmed in the meantime. Overbeek devised an " in vitro " procedure for the quantitative estimation of liver preparations which is based on the fact that explants of bone marrow show an increase of activity through addition of active liver preparations, which manifests itself by a well-marked cell emigration. It could be proved that on the one hand the reaction of the bone marrow is specific for the anti-anaemic principle, and on the other that it acts only on explants of bone marrow but not on those of other organs. Overbeek and his collaborators[41] found, in confirmation of our results, *that highly efficient liver preparations fail completely if bone marrow and blood serum of thyroidectomized animals are applied in the test.* Hence it follows that the liver-activating thyroid principle is immediately active on bone marrow and that we were fully justified in qualifying it as " myelotropic ".

A close study of this thyroid preparation proved that it is highly active in the metabolic test but in a contrary sense to the one to which we were accustomed when dealing with thyroid preparations. A very considerable decrease is noticeable after its administration in the oxygen consumption as well as of the CO_2-production of rats and dogs. The analysis showed that we were dealing with two thyroid substances of importance for heat formation, which we called thermothyrins, to be discussed fully in Chapters XII and XIII. It was ascertained that these had nothing in common with the activation of the anti-anaemic liver principle and that they have to be separated from our " myelotropic hormone ". We are about to produce, after removal of the thermothyrins, the real effective substance from the " myelotropic " hormone. The difficulty of having to deal with apparently infinitesimal quantities is offset by the advantage that we possess an excellent test procedure for its proof on thyroidectomized rabbits, anaemized in conformity with Gottlebe's method.

40. Overbeek, G. A., Gaillard, P. J., and de Jongh, S. E.: *Schweiz. Med. Woch.* (1938), **68**, 711.

41. Gaillard, P. J., Overbeek, G. A., and Tan Hong Yam: *Arch. Int. Pharmacodyn.* (1940), **64**, 33.

CHAPTER IV

THE RELATIONSHIP BETWEEN THE THYROID GLAND AND ANTI-ANAEMIC SUBSTANCES OF STOMACH AND LIVER

THE above-mentioned experiments supplement Mendershausen's[36] pathological-anatomical observation that an atrophy of the thyroid gland exists in pernicious anaemia. They raised in us the idea that here was not a matter of a secondary change of this endocrine gland conditioned by anaemia, but rather that its functional disorder was one of the causes of the origin of this illness. In any case we saw that the thyroid gland or one of its active substances was absolutely indispensable for the efficacy of the anti-anaemic liver principle with regard to saponin-collargol-anaemia. On the other hand, it was clear that the absence of the thyroid function alone can never produce the picture of pernicious anaemia, because both total extirpation of the thyroid in animal experiments and serious cases of thyreopriva cachexia lack its characteristic criteria. That occasionally cases occur in which a hypothyreosis associates with pernicious anaemia, as in the above-mentioned cases of Boros and Czoniczer[34] and Vannotti,[35] speaks for the fact that a combination of several toxic agents as a whole condition the disease; an assumption which, as we reported elsewhere, was made by Mendershausen.[36]

We achieved further clarification of the conditions by experiments analyzing whether thyroid activity was also necessary for the efficacy of the anti-anaemic stomach principle, which, as Sturgis and Isaac[42] and Sharp[43] demonstrated, not only reaches but often exceeds the anti-anaemic effectiveness of the liver. This investigation appeared somewhat unjustified in view of the fact that it is generally accepted that the active substances of stomach and liver are identical and that the actual anti-anaemic principle is formed by the stomach and merely stored by the liver. That this assumption was wrong was demonstrated when we investigated an effective stomach preparation (Perstomin *Richter*) on a thyroidectomized rabbit, anaemized by Gottlebe's method. We established *that, contrary to liver preparations, this preparation was fully effective on thyroidectomized animals as well.* (See Diagram 5.)

Hence it follows that the anti-anaemic substances of stomach and liver can never be identical: *while the active substance*

of the stomach can be active by itself, the liver principle depends for its activation on the thyroid gland.

This result was of great importance for our later experiments. It is known that achylia in man or total extirpation of the stomach in an animal experiment does not lead to *hyperchromic* anaemia, similar to the pernicious kind. In this respect the animal experiments of Svend Petri[44] and his collaborators are most relevant; after total extirpation of the stomach or of stomach and duodenum portions in dogs, which they kept alive for 16 months, these workers could observe only a regularly developing *hypochromic* anaemia. This harmonizes well with the clinical observation that even serious achylia and extensive stomach resection are in the majority of cases not accompanied by pernicious anaemia. (Fleischhacker and Klima.[45]) At times, however, it was observed that a hyperchromic macrocytic anaemia associated itself with achylia which had already existed for some time. All this points to the fact that in addition to an impaired function of the stomach, there must be some other factor without which pernicious anaemia does not take place.

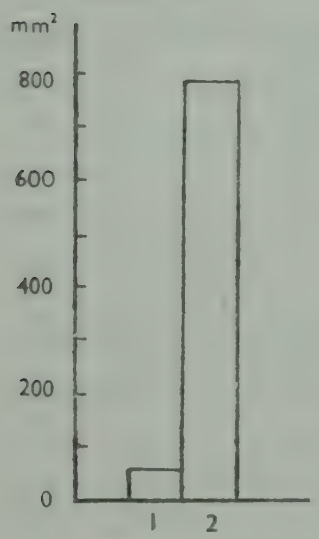


DIAGRAM 5.

1. Effect of liver preparation on *thyroidectomized* anaemized rabbit.

2. Effect of stomach extract on *thyroidectomized* anaemized rabbit.

This assumption is upset by Bence,[46] who claims to have observed over a period of one and a half to two years the development of a hyperchromic macrocytic anaemia on three gastrectomized pigs. Table IV, which shows the most important data of Bence's investigations, demonstrates clearly that this conclusion finds no support in the results of his experiments. The anaemia, which existed for one and a half to two years after gastrectomy, cannot be considered objectively as representing the hyperchromic macrocytic type, because the colour index shows a substantial decrease in all three experiments and because the average erythrocyte diameter also suffers a depression in all cases.

TABLE IV
Results of Bence's Experiments.

Date	No. of Blood Corpuscles. Mill.	Hb. %	Colour Index	Average diameter of erythrocytes μ	Date	No. of Blood Corpuscles. Mill.	Hb. %	Colour Index	Average diameter of erythrocytes
Before Gastrectomy.					After Gastrectomy				
15.10.32	7·2	100	1·0	5·1	18.4.34	3·55	41	0·82	3·8
27.5.32	7·6	100	1·0	4·9	4.1.34	4·94	46	0·70	4·6
27.5.32	8·2	100	1·0	4·8	7.12.34	5·75	55	0·79	4·0

The fact that a few months after gastrectomy the colour index indicated still lower values but later on showed an increase is not sufficient to allow us to speak of a hyperchromic anaemia. Bence's final conclusion that gastrectomy alone leads to the development of pernicious anaemia, a conclusion contradicted by all clinical observations, was intended as a confirmation of his earlier experiments. In these, he did not succeed in obtaining effective preparations from the liver of pigs which had been gastrectomized six months earlier. He, therefore, concluded that the liver principle is produced by the stomach. If this were the case, stomach resection alone, in accordance with Bence's views, would suffice to bring about pernicious anaemia. However, we have seen that the theory of identity of the two active substances is as little defensible as Bence's conclusion that a hyperchromic macrocytic anaemia in pigs is brought about after stomach resection. His discovery, that the liver of gastrectomized pigs does not furnish any active substances, is likely to have been conditioned by operative damage to the liver, which, in fact, finds expression in the pathological-anatomical condition of the operated animals.* Should gastrectomy alone suffice for the formation of pernicious anaemia, as Bence believes, one is led to wonder why the latter does not develop earlier than after an interval of two years, bearing in mind the evidence of Bence's own experiments that the liver does not contain any active substances six months after the stomach resection.

As we see, there are contradictions not only between the results and conclusions of Bence, but also among the results themselves, which would have to be removed before a proper valuation of Bence's experiments could be made.

If we bear in mind only the actual findings, everything points to the existence of a further toxic agent of importance for the pathogenesis of pernicious anaemia. Both the findings of Mendershausen and our own experiments point in the first place to the under activity of the thyroid gland.

The fact that the liver exerts its influence only when its effective substance is activated by the thyroid, whereas the stomach principle has an independent effect of its own, explains why a

* In Case 1: liver organically grown together with the spleen so that they can only be separated by a knife; in a few places the liver had also grown into the intestine, was light grey, diminished and of firm consistency.

In Case 2: liver fundamentally changed in some parts. The augmented interstitium penetrates the acini, almost smashing them, and forms there a multitude of round cells as a sign of an advanced interstitial hepatitis.

In Case 3: liver of firm consistency. Microscopically serious alterations in several places; the increased interstitium penetrates the acini, forming there a multitude of round cells (hepatitis interstitialis).

deteriorated or missing function of the thyroid gland alone never leads to pernicious anaemia; the stomach function is concerned only with a normal development of the formation of blood. The missing stomach function alone does not produce pernicious anaemia either, because the liver possesses full efficacy if the thyroid function is intact.

We can, therefore, draw the following conclusion with regard to pernicious anaemia: *the conditions necessary for the formation of pernicious anaemia are created by deficiency of the stomach function and a simultaneous lack of thyroid activity.*

Such an assumption would explain many an enigma in the pathogenesis of pernicious anaemia. First of all the fact that there are cases of achylia (also of the histamine refractory type) without blood changes, that both hypochromic and hyperchromic anaemias can be associated with achylia and, finally, that achylia can precede all other symptoms of pernicious anaemia by a considerable span of time. If, on the grounds of the reported relationship between the thyroid and the anti-anaemic principle of stomach and liver, we assume that pernicious anaemia is conditioned by a deficient stomach and thyroid function, we need not be surprised that even severe achylia may not lead to pernicious anaemia but may often precede it.

In order to examine this hypothesis, we had to investigate whether an anaemia, similar to the pernicious type, can be produced in animals by a simultaneous elimination of the stomach and of the thyroid function.

42. Sturgis, C. C., and Isaac, R.: *J. Amer. Med. Assoc.* (1929), **93**, 747.
43. Sharp, E. A.: *J. Amer. Med. Assoc.* (1929), **93**, 749.
44. Petri, Svend: *Folia Haematologica.* (1935), **54**, 150.
45. Fleischhacker and Klima: *Z. klin. Med.* (1936), **129**, 227.
46. Bence, J.: *Z. klin. Med.* (1936), **130**, 275.

CHAPTER V

EXPERIMENTAL PERNICIOUS ANAEMIA

Our first experiments to produce hyperchromic anaemia by simultaneous damage to the stomach function and elimination of the thyroid were made on white rats. As stomach resection is impossible on these animals, they were only opened and the mucous membrane painted with 5 per cent trichloracetic acid.

Four animals survived. Operations were performed on the stomachs of the first two and on the thyroids of the other two. These operations were failures. After the second operation, however, as Diagram 6 shows, the number of the blood corpuscles dropped considerably, and in three cases the colour index rose. This condition lasted only two to three weeks, due, as can be seen from the autopsy, to a regeneration of the mucous membrane of the stomach.

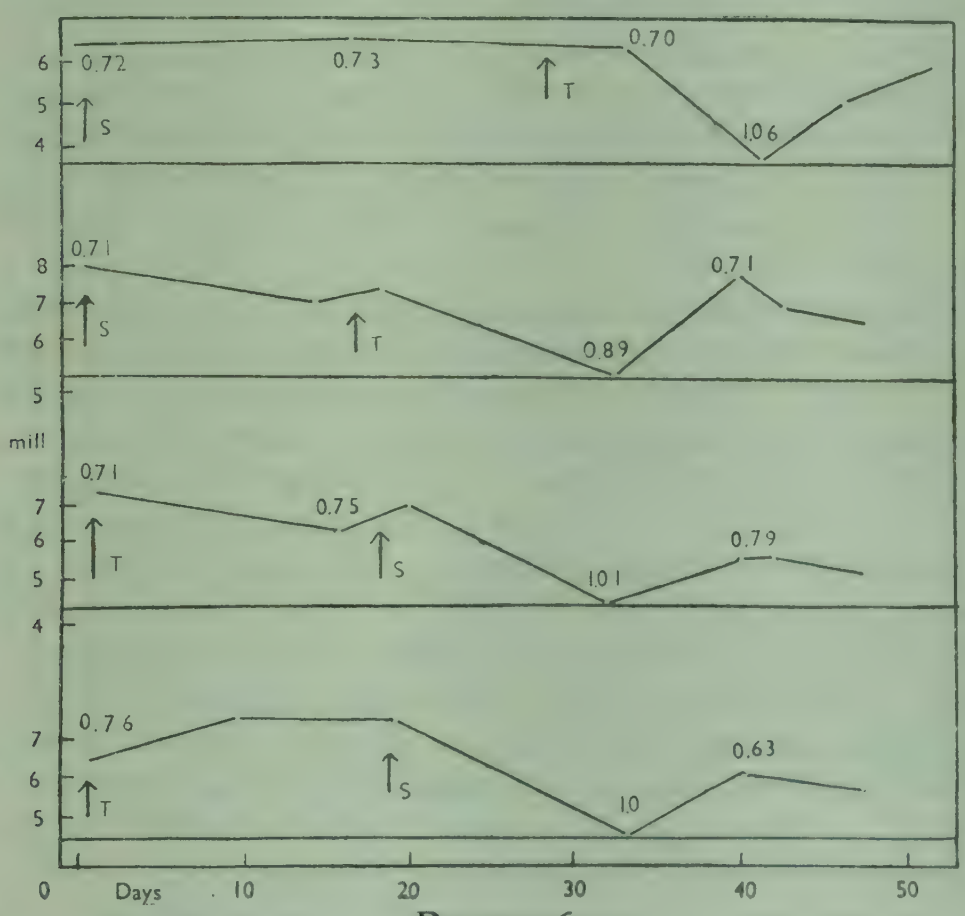


DIAGRAM 6.

Variation of blood corpuscles and colour index in rats. The number of blood corpuscles is shown on left and the graph corresponds to these. The numbers at the various points on the graph show the colour index. S=Stomach operation. T=Thyroidectomy.

These experiments were confirmed by Overbeek and Vissmans,[46a] who succeeded in producing in thyroidectomized rats a hyperchromic anaemia which was resistant against liver therapy, by a diet free from the "extrinsic factor".

Repeating our experiments on rats with cauterization of the mucous membrane of the stomach, Overbeek and Gaillard[46b] were able to prove a speedy regeneration of this membrane. In two cases, however, they also obtained serious anaemia after the double operation.

After these encouraging preliminary experiments, we continued our investigations on dogs and rabbits.

(*a*) Experiments on Dogs

We owe the discovery, that in achylia (which is one of the contributory factors in respect of pernicious anaemia) it is not a matter only of a lack of hydrochloric acid and pepsin, to the extensive investigations made by W. B. Castle and his collaborators, Heath, Strauss, and Townsend.[47]

These investigations led to the finding that the gastric juice of healthy persons, freed from its enzymes, allowed the effective principle to be formed just the same as if the enzymes could exert their influence on the nutritive factor. Hence it follows that in the normal stomach secretion there must exist a hitherto unknown factor of its own, which, independently of pepsin and rennin, forms the anti-anaemic principle out of certain nutritive constituents.

Without knowing any details about the formation of this endogenous Castle's factor, it may be assumed that this portion of the stomach secretion is formed by the glands of the mucous membrane of the stomach. We therefore strove to destroy them completely. For this purpose, we first reduced the stomach to about 1/3. We did so by tying up the vessels along the large curvature, sparing the spleen artery. We then fixed two large stomach clamps parallel to the large curvature and removed the largest portion of the stomach fundus through a longitudinal section between them. Sterile cloths were applied to the intersecting edges, the stomach clamp was released. and the remaining stomach fragment, which now formed a bag between cardia and pylorus, was opened up. After removal of any possible secretion, we damaged the mucous membrane chemically.

This was done in the following way: We cauterized the mucous membrane with 10 per cent trichloracetic acid and after about five minutes' action washed out the stomach with a solution of sodium chloride or, in some cases of acid cauterization, we preceded with

a paint of 5 per cent NaOH. After the washing out with the sodium chloride solution, we cauterized with 10 per cent trichloracetic acid.

There was no special advantage in this combined treatment, for it did not work. Even so after some time a regeneration of the mucous membrane of the stomach took place. We saw the most pronounced and most lasting pernicious anaemia in an animal whose mucous membrane we damaged by this combined treatment. The painting must be done thoroughly up to the cardia and in most cases we painted the mucous membrane of the upper duodenum beyond the pylorus as well. The stomach was then sutured.

Total thyroidectomy was performed in the usual way, preserving two parathyroids.

At the beginning we lost a few animals, because we damaged the mucous membrane excessively, so that the stomach was perforated. If, however, the damage was not extensive enough, it remained ineffective, or an early regeneration of the mucous membrane and "cure" of the pernicious anaemia followed. In two such cases we succeeded in reproducing the anaemic blood picture by a second stomach operation.

Because of the danger of an early regeneration, it was preferable to undertake the stomach operation after thyroidectomy. In the experiments in which thyroidectomy took place two to three weeks after the stomach operation, we never saw a change of the blood picture. When, however, thyroidectomy was performed before or shortly after the cauterization of the mucous membrane of the stomach, the operation was mostly successful and the blood alteration a lasting one. This points to the fact that regeneration of the damaged mucous membrane of the stomach is accelerated when the thyroid gland is active.

Below, we show the blood alterations in animals which have undergone double operations and where such action proved to be effective.

Dog 24

Date	Erythrocytes Mill.	Hb. %	Colour Index	Remarks
2 XII 38	4·6	94	1·00	Normal
3 XII 38	—	—	—	Thyroidectomy
6 XII 38	4·6	82	0·88	
7 XII 38	—	—	—	Resection of stomach Two-thirds removed. Alkaline and acid cauterization
10 XII 38	3·6	89	1·23	
19 XII 38	3·5	78	1·11	
5 I 39	3·4	80	1·17	
18 I 39	3·1	83	1·33	
30 I 39	3·1	75	1·20	

On February 1st, 1939, the animal succumbed to a second laparotomy for total extirpation of the remaining stomach fragment.

Dog 21

Date	Erythrocytes Mill.	Hb. %	Colour Index	Remarks
6 XI 37	5·5	92	0·84	Normal
7 XI 37	—	—	—	Stomach resection. Two-thirds removed. Acid cauterization
20 XI 37	6·2	107	0·85	Thyroidectomy
25 XI 37	—	—	—	
30 XI 37	4·0	86	1·07	
6 XII 37	3·8	82	1·08	
20 XII 37	4·0	78	0·98	
2 I 38	4·0	70	0·88	
3 I 38	—	—	—	Stomach opened. Mucous membrane of stomach regenerated. Cauterization with alkali and acid.
7 I 38	2·1	68	1·50	

Dog 22

Date	Erythrocytes Mill.	Hb. %	Colour Index	Remarks
20 XI 37	5·2	93	0·86	Normal
21 XI 37	—	—	—	Stomach resection. Two-thirds removed. Acid cauterization
25 XI 37	—	—	—	Thyroidectomy
4 XII 37	4·4	110	1·24	
13 XII 37	4·4	102	1·16	
27 XII 37	5·2	117	1·12	
30 I 38	4·1	90	1·10	
19 II 38	3·6	92	1·27	
13 III 38	4·6	96	1·14	
20 V 38	3·6	85	1·18	
15 VI 38	3·8	92	1·21	
17 VI 38	3·7	88	1·19	
19 VI 38	3·5	86	1·22	
21 VI 38	3·5	85	1·21	
23 VI 38	3·9	81	1·04	
29 VI 38	4·5	92	1·00	
5 VII 38	4·6	85	0·91	
18 VIII 38	4·5	90	1·00	
5 X 38	4·4	92	1·04	
23 XI 38	4·5	82	0·93	
8 II 39	4·1	82	1·00	
16 V 39	4·0	72	0·90	
22 XI 39	4·2	77	0·93	
30 XI 39	2·0	—	—	Stomach operation. Mucous membrane of stomach regenerated. Acid cauterization
4 XII 39	4·6	88	0·94	
7 XII 39	3·3	75	1·17	
17 I 40	3·9	87	1·10	
24 I 40	4·1	102	1·06	
15 II 40	3·7	70	—	
29 III 40	4·2	65	0·77	
5 IV 40	4·2	69	0·84	Free HCl : 48. Total acidity : 62
14 VI 40	5·1	78	0·78	

Dog 33
Thyroidectomy, June 1936

Date	Erythrocytes Mill.	Hb. %	Colour Index	Remarks
30 XI 37	5·4	98	0·90	
4 XII 37	—	—	—	Stomach resection. Two-thirds removed. Alkaline cauterization
10 XII 37	4·7	98	1·04	
15 XII 37	4·2	89	1·06	
20 XII 37	4·9	83	0·85	
24 XII 37	5·0	99	0·99	
5 I 38	—	—	—	Stomach operation. New cauterization of the mucous membrane with alkali and acid
10 I 38	3·7	82	1·10	
20 I 38	3·8	83	1·09	
5 II 38	3·8	88	1·16	
15 II 38	5·5	100	0·91	
2 III 38	5·4	94	0·87	

The animal lived for a year and a half without its thyroid prior to the first stomach operation and had a normal blood picture. After partial stomach resection and cauterization of the mucous membrane, a transitory moderate anaemia developed with an increased colour index. The success of the second damage to the mucous membrane was somewhat greater but did not last long either, because, about six weeks later, the normal number of erythrocytes was reached again and the colour index dropped to its original value.

Dog 47

Date	Erythrocytes Mill.	Hb. %	Colour Index	Remarks
5 III 38	5·0	90	0·90	Normal
9 III 38	—	—	—	Resection of stomach. Alkali and acid cauterization
16 III 38	4·3	80	0·93	
20 III 38	5·2	83	0·80	
24 III 38	—	—	—	Thyroidectomy
9 IV 38	3·9	92	1·18	
29 IV 38	4·5	93	1·04	
11 V 38	4·5	92	1·02	
11 VI 38	4·6	110	1·20	
29 VI 38	4·4	94	1·07	
30 VII 38	4·2	86	1·07	
18 VIII 38	4·3	88	1·02	
5 IX 38	4·0	82	1·02	
5 X 38	1·3	47	1·80	
6 X 38	1·3	48	1·84	
7 X 38	1·2	49	2·04	
8 X 38	1·0	47	2·35	2·5 cc. myelotropic hormone
9 X 38	1·7	47	1·38	
10 X 38	1·5	46	1·53	
11 X 38	1·1	45	2·00	5 cc. myelotropic hormone
12 X 38	1·4	43	1·53	
13 X 38	1·2	44	1·83	
14 X 38	1·3	41	1·57	
17 X 38	1·1	43	1·95	
18 X 38	0·9	42	2·33	
19 X 38	—	—	—	4cc. liver preparation "Campolon"
20 X 38	1·1	42	1·90	
21 X 38	1·5	49	1·63	
22 X 38	1·5	48	1·60	
23 X 38	1·7	48	1·70	
1 XI 38	1·8	55	1·52	
5 XI 38	2·2	61	1·38	
10 XI 38	2·8	61	1·09	
17 XI 38	2·8	61	1·08	
30 XI 38	3·0	72	1·20	
8 XII 38	3·7	80	1·08	
30 I 39	3·5	82	1·17	
6 II 39	3·8	75	0·98	
16 III 39	4·3	78	0·90	
11 IV 39	4·6	85	0·92	
16 V 39	4·0	68	0·87	
7 VI 39	4·4	75	0·85	
10 VII 39	4·1	83	1·01	
11 VII 39	—	—	—	Onset of tetanus, cured by 20E. Parathormone + 0·4g. $CaCl_2$ intravenously
1 VIII 39	—	—	—	
25 X 39	3·8	69	0·90	
21 XI 39	—	—	—	Onset of tetanus. 50E Parathormone. Cramp cured
22 XI 39	—	—	—	Dead

The resection had the following results: (1) The mucous membrane of the stomach was completely regenerated, as confirmed by microscopic investigation. (2) The parathyroids were strongly vascularized, the connective tissue was slightly thickened at the expense of the gland cells; otherwise the picture was normal. (3) There was nothing abnormal to be seen on the liver, either

macroscopically or microscopically. (4) The thymus, on the contrary, was completely degenerated. Macroscopically its place was filled with small cysts, which contained a yellow liquid. Microscopically, only the interstitial structure of the connective tissue of the gland could be recognized.

It is interesting to note that as a result of this experiment, soon after the double operation, a moderate but markedly hyperchromic anaemia developed, but *the actual pernicious anaemia occurred in September* 1938, *after a latent period of six months; in October it reached its culminating point with* 0.9 *mill. erythrocytes and a colour index of* 2.3. Such a blood picture is rare even in human pathology. This serious condition lasted for about two months, gradually improving, and the number of the erythrocytes in April 1939 rose to 4–4.5 mill. with a normal colour index of approximately 0.80. It is difficult to decide if this turn for the better was a result of the administration of the myelotropic hormone in conjunction with a large dosage of liver preparation. At all events, after the hormone injections, the blood picture showed transitory improvement, noticeable in the increase of the number of blood corpuscles and drop of the colour index. A lasting improvement took place only after an injection of the liver preparation. No relapse occurred because the mucous membrane regenerated, which was demonstrated by the obduction. It is difficult to explain why this serious pernicious anaemia took place after a latent period of six months. In my view, the accidental destruction of the thymus may have been an important factor. An interesting observation lends support to this assumption.

As it is much easier to perform gastrectomy on dogs less than a year old than on older ones, we operated exclusively on very young dogs in all our experiments that followed hereafter. The result was that in very young dogs neither total gastrectomy nor partial resection and damage to the mucous membrane combined with thyroidectomy led to a hyperchromic anaemia.

This coincides with the fact that spontaneous pernicious anaemia occurs in adults but never in children.

We are about to clarify a possible participation of the thymus in experimental pernicious anaemia.

The total operative removal of the thymus in dogs is so done that the sternum of an animal receiving artificial respiration is split in the middle line with an electrical circular saw; the thymus can then be easily removed from the opened-up thorax without any bleeding. The sections of the sternum are united with silver wires, and the wound stitched as usual. After removal of the tracheal cannulae, the trachea is closed with a catgut suture.

Another great difficulty arose from the fact that, after

thymectomy, the removal of the thyroid gland always led to tetany, with one exception, in spite of the fact that, as usual, we conserved two parathyroid glands; this may be connected with the known influence of the thymus gland on calcium metabolism. (Leites.[48]) It is remarkable that this dog, No. 47, with a degenerated thymus, was the only one among our many thyroidectomized dogs to die of tetany. We still hope for the successful performance of our double operation on thymectomized dogs, as this appears important in view of the fact that the thymus has been considered to be a blood-forming organ. (Trendelenburg.[48a])

It is interesting to note that, after the very promising finding of Eisler, Hammarsten, and Theorell,[49, 50] they succeeded, with the aid of H. Theorell's cataphoresis, in proving the existence of two principles in active liver preparations, which only together bring about erythropoiesis and reticulocytosis in a rabbit, anaemized after Eisler's[51] method with hydroxylamine. It was shown that one of these components, the so-called R-substance, can be substituted by thymonucleic acid so that the E-substance of liver preparations (which, in cataphoresis, migrates to the negative side, and when applied alone has no measurable effect on erythropoiesis or reticulocytosis) becomes highly effective when administered together with sodium thymonucleate.

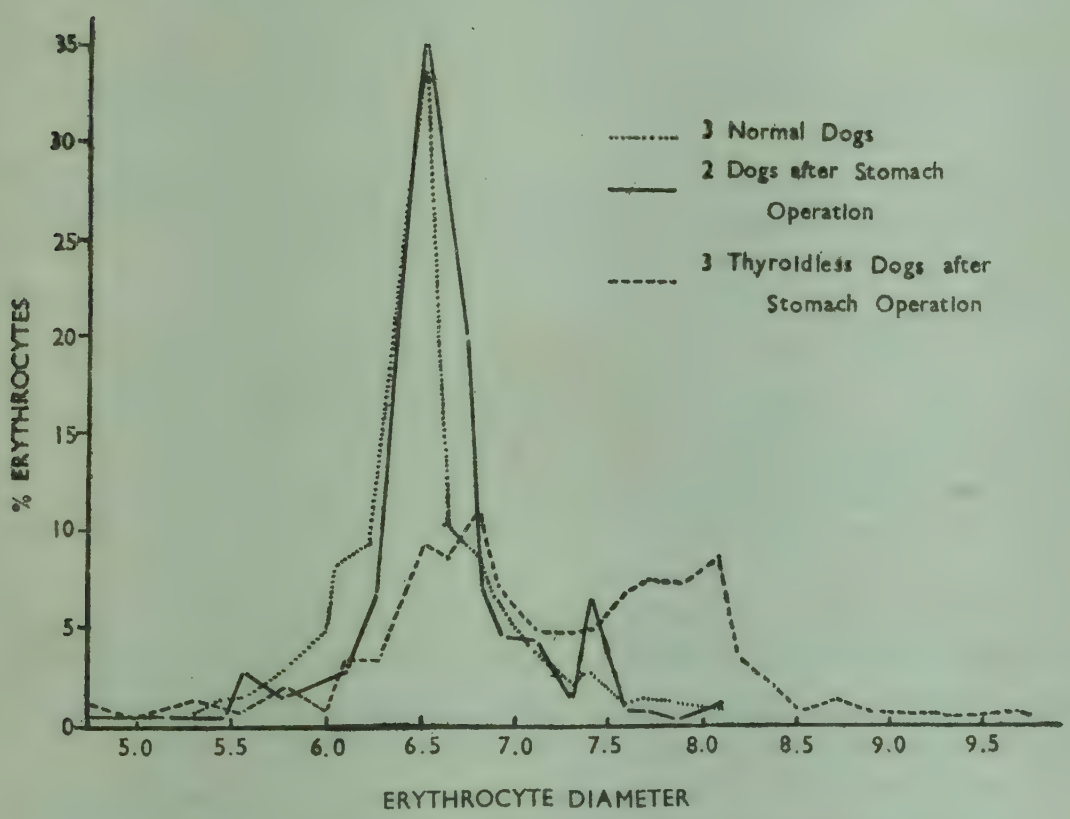


DIAGRAM 7.

Variation of erythrocyte diameter after stomach operation in normal and thyroidectomized dogs.

Finally, Diagram 7 demonstrates the alteration of the diameters of the erythrocytes, which makes it clear that only the three animals which underwent double operations showed macrocytic anaemia, whereas the erythrocytes of two dogs that showed anaemia after the stomach operation were of normal size.

(*b*) Experiments on Rabbits

On rabbits we opened the stomach and, without reducing it, cauterized the mucous membrane with 5 per cent trichloracetic acid, washed it out with a solution of sodium chloride, and sutured it up again. Of the rabbits subjected to the double operation only five survived. Hyperchromic anaemia was produced in four of these rabbits, whilst the operation remained unsuccessful on the fifth. We demonstrate the development of these four experiments in the following diagrams:

Diagram 8.

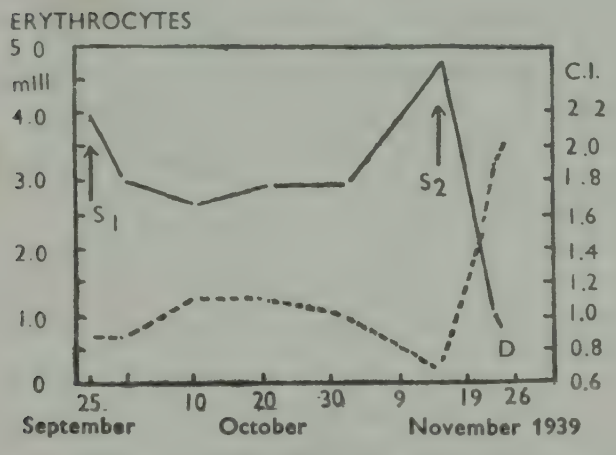


Diagram 9.

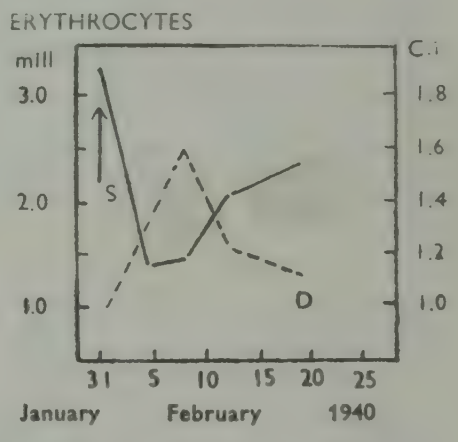


Diagram 8: Rabbit No. 1. Thyroidectomy on 10th May, 1939.
S_1 = First stomach operation on 25th September, 1939.
S_2 = Second stomach operation on 16th November, 1939.
D = Death of rabbit.
—— Erythrocytes in millions per c.mm.
- - - - Colour index.

Diagram 9: Rabbit No. 18. Thyroidectomy in October 1939.
S = Stomach operation on 31st January, 1940.
D = Death of rabbit.
—— Erythrocytes in millions per c.mm.
- - - - Colour index.

In Diagram 11, we show an experiment, in which serious hyperchromic anaemia took place promptly after the double operation but disappeared quickly after a repeated intake of large doses of our " myelotropic hormone ".

If we survey the result of our experiments, we see that on a thyroidectomized animal an extensive damage to the mucous membrane of the stomach leads regularly to the development of a hyperchromic macrocytic anaemia. It is similar to the pernicious

type, which sometimes reaches such high degrees as to be extremely rare even in human pathology, particularly so since the introduction of liver therapy.

DIAGRAM 10.

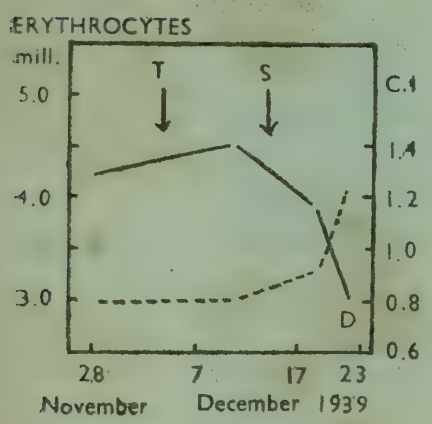


DIAGRAM 11.

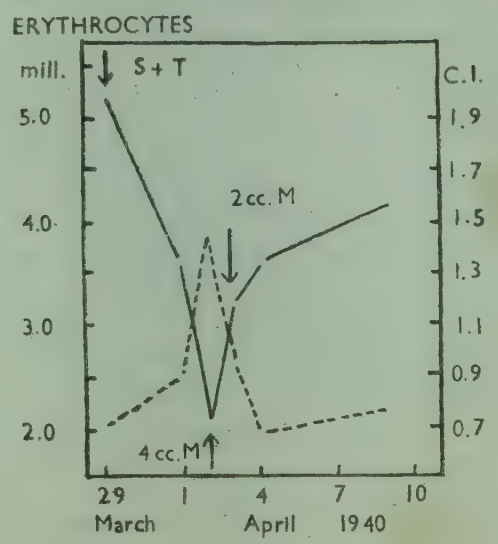


DIAGRAM 10: Rabbit No. 7.
T = Thyroidectomy on 4th December, 1939.
S = Stomach operation on 12th December, 1939.
—— Erythrocytes in millions per c.mm.
- - - - Colour index.

DIAGRAM 11: Rabbit No. 10.
S + T = Thyroidectomy and stomach operation on 29th March, 1940.
M = Intake of myelotropic hormone.
—— Erythrocytes in millions per c.mm.
- - - - Colour index.

(c) RELATIONSHIP BETWEEN EXPERIMENTAL AND SPONTANEOUS PERNICIOUS ANAEMIA

We do not claim that experimental pernicious anaemia fully explains the pathogenesis of this disease. It seems probable, however, that it is of importance for its clarification.

Our findings, first reported at the International Congress of Physiologists in Zurich, in 1938, have not only been confirmed in the meantime, but received an interesting addition, which points to the not only superficial but substantial similarity between our experimental anaemia and that of man.

We mentioned earlier that Overbeek and his collaborators[40] were able to demonstrate that therapeutically active liver preparations effect a cell migration in bone marrow explants which is suitable for the quantitative evaluation of liver preparations. Overbeek and his collaborators[41] reported that, in accordance with our results, these liver preparations are ineffective if we use in this experiment bone marrow and blood serum of thyroidectomized

animals. In a recent report of Overbeek and Gaillard,[46b] this result was confirmed in three further experiments and at the same time it was proved that it suffices if only one of the two animals which supply bone marrow or plasma for the test is deprived of its thyroid. Still more important, however, is a further discovery of these authors, that *bone marrow explants of patients suffering from pernicious anaemia react to an addition of active liver preparations as little as bone marrow of thyroidectomized animals.* Hence it follows that experimental and spontaneous pernicious anaemia are substantially similar processes.

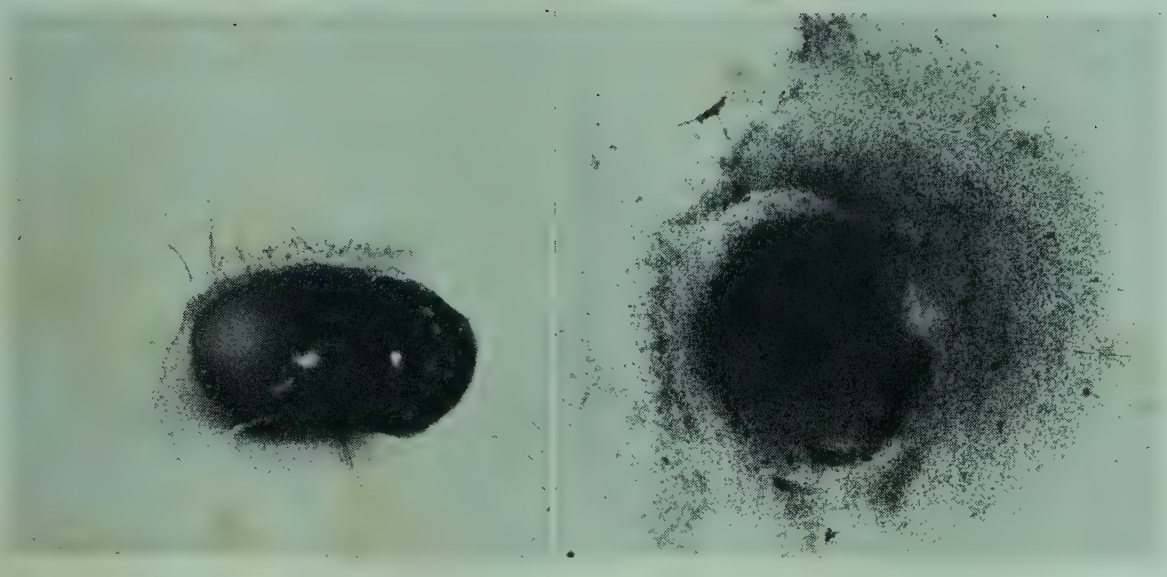
In the Plate (opposite), which has been taken from Gaillard, Overbeek and Tan Hong Yam,[41] we show the difference of the liver effect on the bone marrow of a normal man and of one suffering from pernicious anaemia.

Patients suffering from pernicious anaemia react to liver preparations; thyroidectomized animals do not. This may be explained by that fact that in man there is only an atrophy of the thyroid gland, which probably depresses the reactivity in respect of liver substances without cancelling it; thus an intake of large quantities of the liver principle would direct the blood formation into the proper channels. Our assumption is further strengthened by the clinical experience that in liver refractory cases—which are now rare, due to the introduction of large liver dosages—stomach preparations have proved to be effective.

We hope that further purification and analysis of our myelotropic hormone will soon enable us to establish its effectiveness in experimental pernicious anaemia. Up to now our experiments have shown that liver preparations alone are inactive; but they are active when administered in conjunction with the myelotropic hormone. In a few cases, especially in rabbits, we saw a good effect from thyroid hormone alone, but we failed in dog experiments. Here, further experiments are necessary, in order to clarify the role of the hormone* We owe to the discovery of this new thyroid hormone the way along which we managed to produce experimental pernicious anaemia and to make some contribution to the pathogenesis of this still mysterious disease.

We perceive a fine confirmation of the relation proved by experiments between pernicious anaemia and the thyroid gland in the genealogical tree of a family, which shows that pernicious

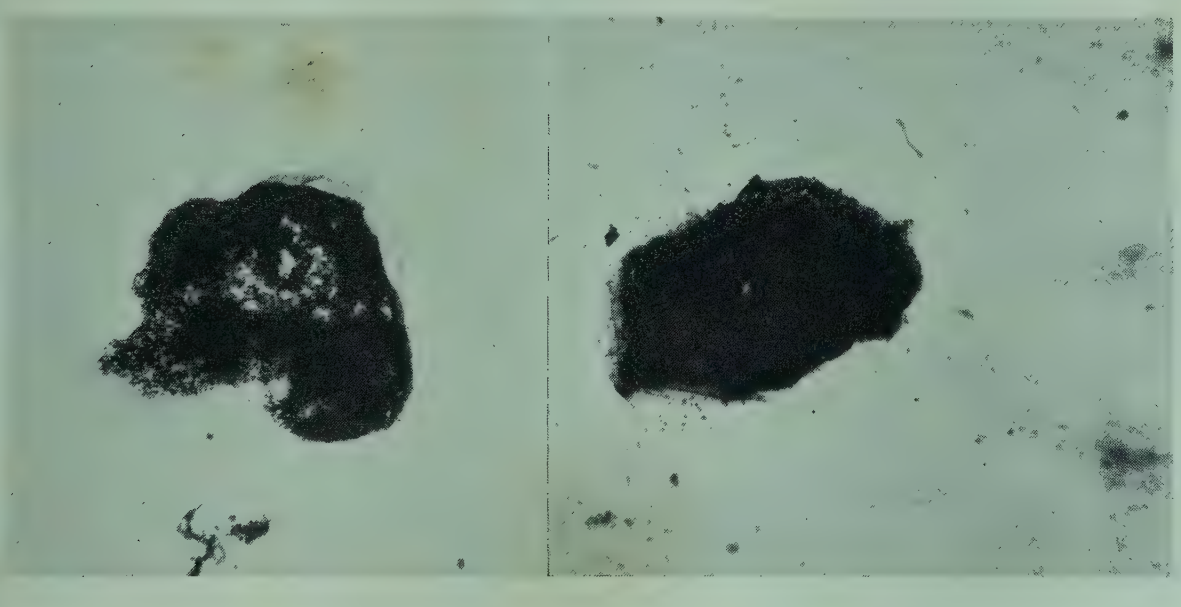
* Overbeek and Gaillard[46b] also missed in their *in vitro* experiments the liver effect on bone marrow explantations of thyroidectomized guinea pigs, which, before the removal of the bone marrow, were treated with the myelotropic hormone. Unfortunately, the plan of these authors to test *in vitro* the effectiveness of our myelotropic hormone on bone marrow explants themselves could not be executed, as there were difficulties in obtaining suitable animal material.



No liver extract added, little migration.

After the addition of liver extract, much migration.

Explants of normal human bone marrow.



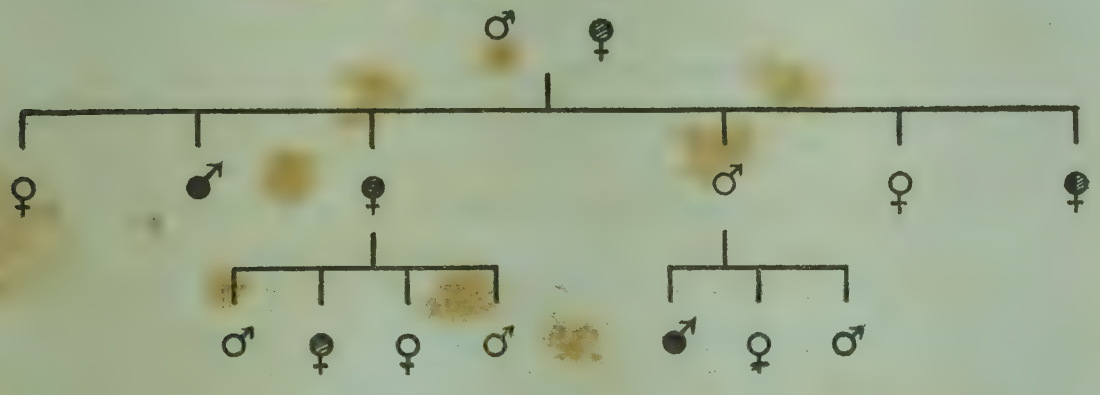
No liver extract added, no migration.

After the addition of liver extract, little migration.

Explants of bone marrow of an untreated patient, suffering from pernicious anaemia.

(By courtesy of Dr. G. A. Overbeek and the *Archives Internationales de Pharmacodynamie et de Thérapie*)

anaemia was inherited partly as Graves' disease and partly as pernicious anaemia; in one case even these diseases, which differ so much externally, appeared simultaneously in one individual. (Diagram 12.)



○ Healthy
◍ Pernicious Anæmia
● Graves' Disease

DIAGRAM 12.

46a. OVERBEEK, G. A., AND VISSMANS, J. B. M.: *Acta. brev. neerl. Physiol.* (1939), **9**, 206.

46b. OVERBEEK, G. A., AND GAILLARD, P. J.: *Arch. Int. Pharmacodyn.* (1942), **67**, 276.

47. CASTLE, W. B., *et al.*: *Amer. J. med. Sci.* (1929), **178**, 784, 764; (1930), **180**, 305; (1931), **182**, 741.

48. LEITES, S.: *Biochem. Z.* (1924), **150**, 183.

48a. TRENDELENBURG: *Die Hormone*, Vol. II, p. 453.

49. EISLER, B., HAMMARSTEN, EINAR, AND THEORELL, HUGO: *Skand. Arch. Physiol.* (1937), **77**, 24.

50. EISLER, HAMMARSTEN, AND THEORELL: *Naturwiss.* (1936), **24**, 142.

51. EISLER, BELA: *Ueber eine experimentell erzeugte chronische Anaemie bei Kaninchen Vortrag.* (Sep).

PART TWO

THYROXIN

CHAPTER VI

THE EFFECT OF THYROXIN ON THE CELL

In the early days of endocrinology, the points of attack of the hormones were considered to be the cells of the end organs; this theory was not doubted. Two of the most important functions of the hormone of the thyroid gland were partly discovered prior to its production in pure form: one, which increases the *function* of the brain by raising the irritability of nervous centres (Duran,[52] Landolt[53]); the other increasing *combustion* in the organs without increasing their functions. In this way it was discovered that the hormone of the thyroid gland possesses the faculty of increasing the basal metabolism.

That it was the task of the thyroid gland to deliver an active substance into the blood, and thereby accelerate combustion in the organ cells, appeared so plausible that no doubt was raised as to the peripheral point of attack. It was all the more surprising, therefore, when, almost simultaneously, several authors reported that thyroxin, which had in the meantime been produced in its pure form by Kendall,[54] was ineffective in Warburg's known apparatus for isolated cells and organ fragments. (Ph. Ellinger,[55] Fleischmann,[56] Singer and Reinwein,[57] Anselmino, Eichler and Schlossmann.[58]) These negative results, strengthened by many a clinical observation, led gradually to the opinion that thyroxin does not act at all on the organ cells themselves, but on the so-called metabolic centres of the mid-brain and that the irritations produced there flow via the autonomic nervous system to the organs in order to increase the basal metabolism in them.

We shall demonstrate in Chapter VIII that this assumption is without any justification and that thyroxin does not increase the basal metabolism, even partially, via the nervous system. We want to discuss first the effect of thyroxin on the combusion in surviving cells and organs and show the reasons why most workers have not succeeded in achieving an increase of combustion due to thyroxin in Warburg's apparatus.

Contrary to these negative results in the Warburg experiment,[59] it struck us that, while investigating the so-called *oxygen-free*

oxidation after Lipschitz[60] and Thunberg,[61] many authors succeeded in achieving an increase of respiration due to thyroxin: Lipschitz and Adler,[62] Neuschloss,[63] as well as Ahlgren,[64] who proved that thyroxin in unexpectedly minute quantities (up to 10^{-18} gm. per c.c.) accelerates the reduction of methylene blue to the same extent as seen for the O_2-consumption in the organism after thyroxin.

A repetition of Ahlgren's experiments brought full confirmation, whereas, with Warburg's apparatus, we failed in hundreds of cases to achieve an increase of O_2-consumption in the various organs of warm-blooded animals due to thyroxin; we did succeed, however, with dinitrophenol.

Due to these experiments, it became clear that thyroxin, although its peripheral effect is beyond doubt, acts on coll oxidation in a different way from dinitrophenol. Bearing in mind this difference, it seems important that in the procedures of Thunberg and Lipschitz the examined cells lack oxygen, whereas in Warburg's process they are rich in O_2. Therefore, we think that the negative results in the latter process are not due to the incapacity of thyroxin, but are caused by the inadequacy of Warburg's method, which is most useful for the analysis of oxidation processes in the cells, but is not suitable for imitating conditions prevailing in the organism.

From the work of Krogh,[65] we know how poorly our organs are supplied with O_2 when resting. In Warburg's apparatus, on the contrary, the living conditions of the cells are plentifully supplied with O_2 and can have only *one purpose*: to consume the combustible substances, found pre-formed, in the cells. The other, no less important, side of metabolism, enzyme action, must in these circumstances recede into the background, because we know that oxidation suppresses it. (Pasteur.[66])

This led to the assumption that thyroxin does not directly accelerate the O_2-consumption but only the preceding enzymatic processes, which through supply of combustible material accelerate secondarily the oxidation processes. Von Euler's[67] success in Warburg's experiment, to achieve an increase of the combustion due to thyroxin by proceeding at a low O_2-concentration of only 5 per cent, coincides with this conception; so do former observations on acceleration of autolysis, i.e., postmortem anaerobic protein splitting due to thyroid substances, e.g. thyroxin. (Abderhalden and Francke,[68] Weil and Landsberg,[69] and Simon.[70]) It could be proved in more than one way that thyroxin brings about an acceleration of combustion only when it has an opportunity of acting on the anaerobic phase of metabolism or on enzymes.

To begin with, we were able to demonstrate that thyroxin is ineffective in the methylene blue experiment, if we suppress enzyme action as far as possible by carrying out the evacuation at ice temperature, before methylene blue is added. This is shown by our experiments in Table V.

TABLE V

Evacuation at room temperature.			Evacuation at ice temperature		
Organ	Thyroxin Concentration	Difference % with added thyroxin	Organ	Thyroxin Concentration	Difference % with added thyroxin
Rabbit brain (minced)	10^{-17}	+27	Rabbit brain (minced)	10^{-12}	+ 2
	10^{-13}	+32		10^{-13}	+ 2
	10^{-9}	+32		10^{-14}	—15
	10^{-12}			10^{-14}	0
	10^{-14}	+19		10^{-16}	— 6
Rabbit muscle (minced)	10^{-11}	+16	Frog muscle (minced)	10^{-12}	+14
	10^{-11}	+14		10^{-13}	+14
				10^{-13}	+ 5
Frog muscle (minced)	10^{-14}	+30		10^{-13}	+ 4
	10^{-14}	+35		10^{-13}	+ 4
	10^{-13}	+33		10^{-13}	— 4
	10^{-13}	+33			

On the other hand, we succeeded in proving in a positive sense that O_2-deficiency—usually lacking in Warburg's experiment—is a preliminary condition for the thyroxin effect. If, before the actual respiration period, we allow the cells to remain for 20–30 minutes *under O_2-deficiency with thyroxin*, we can see that, in this case, thyroxin proves to be highly effective for combustion. The following experiments in Table VI demonstrate it clearly.

TABLE VI

Organ	O_2-consumption in cc. per 30 min. after 0_2-deficency of 30 min.		Increase %
	Without	With	
	Thyroxin		
Rabbit muscle (minced)	32	43	34
	21	29	38
	42	52	23
	55	67	21
	53	74	39
	55	64	13
	73	85	16
	70	82	17
Rabbit brain (minced)	112	135	20
	233	285	22

This also confirms that thyroxin becomes effective only if given the opportunity of acting on the anaerobic phase of metabolism. That it is really a matter of accelerating enzymatic processes can be demonstrated, as follows:

It is known that enzyme action is hindered by respiration, which means that both these processes are interconnected by a

chemical reaction, the so-called Pasteur's reaction.[66] Shigeru Toda[71] discovered that the ethyl ester of hydrocyanic acid (ethylcarbylamine: $C_2H_5N=C$) possesses the effect of selectively inhibiting Pasteur's reaction without influencing the oxidations or enzyme actions themselves. If it is correct to assume that thyroxin primarily accelerates the enzymatic processes, then it must become active also when the O_2-supply is plentiful, if we poison the cells with ethylcarbylamine and thereby create a condition in which enzyme action and combustion develop simultaneously and side by side.

That this is really the case could be proved in the organs examined by us, with the exception of the muscles in which ethylcarbylamine is speedily disintegrated by lactic acid, as shown by Table VII.

TABLE VII

Ethylcarbylamine Experiment.

Organ	O_2-consumption in mm.³ per 15 min. after addition of $C_2H_5N=C$		Increase %
	Without	With	
	Thyroxin		
Rabbit brain (minced)	35	41	17
	41	49	19
Rabbit liver (minced)	54	62	14
Rabbit kidney (sliced)	$Qo_2=9$	$Qo_2=10$	11
Guinea pig liver (sliced)	$Qo_2=3.5$	$Qo_2=4.3$	25
	$Qo_2=4.5$	$Qo_2=5.2$	15

Warburg found that every damage to the cell, whether due to too high or too low a temperature, a disturbed equilibrium of the ions, or oxygen deficiency, etc., leads to inhibition of Pasteur's reaction, similar to that caused by ethylcarbylamine poisoning. Now we understand why in Euler's experiments with only 5 per cent O_2, in our own experiments with an anaerobic preliminary period, and in the anaerobic arrangement of Thunberg and Lipschitz, the effect of thyroxin makes itself felt, whereas it is lacking in Warburg's experimental procedure under a maximum supply of O_2. Everything that inhibits the Pasteur-reaction by cell damage creates favourable conditions for thyroxin effectiveness. That Haarmann,[72] contrary to ourselves, also saw powerful increases of O_2-consumption due to thyroxin under aerobic conditions in Warburg's apparatus can be explained by the fact that he undertook his experiments on organs of warm-blooded animals at a temperature of 28°C., which, of course, implies an injury to the cells. We shall discuss later why thyroxin also effects an increase of combustion in undamaged cells of the organism.

In this connection we should like to report on experiments, made under anaerobic conditions, not on organ cells but on yeast, which most impressively demonstrate the effect of thyroxin on cells. An increase of respiration is best obtained by damaging the oxidation system of the cells by small doses of hydrocyanic acid or addition of ethylcarbylamine, which inhibits Pasteur's reaction.

TABLE VIII
Yeast

O_2-consumption in cu. mm. per 60 min.		Increase %	Substance added
Without	With		
Thyroxin			
183	295	61	KCN M/600
63	112	77	KCN M/300
81	157	93	KCN M/600
103	145	40	$C_6H_5N=C$ M/500

Another question was: Which anaerobic process is accelerated in the cell by thyroxin?

The experiments recounted in the older literature, that the increase of combustion conditioned by the thyroid goes hand in hand with an increase of protein combustion—which is not the case in heterogeneous increases of metabolism, like muscular activity for instance—strengthen the belief that this is a matter of splitting protein substances. The above-mentioned fact relating to the increase of autolysis, i.e., postmortem protein splitting due to thyroxin, confirms this and finds support also in our experiments, in which we analyzed the effect of thyroxin not only as far as respiration was concerned but also upon the formation of ammonia, by Warburg's method.[76] From Table IX we see that the increase of O_2-consumption and NH_3-formation run parallel to one another, and an increase of NH_3 was noticeable only in those experiments where an increase of respiration was made possible by an anaerobic preliminary period.

TABLE IX

Organ	Increase due to Thyroxin		Remarks
	Respiration %	NH_3 formation %	
Rabbit muscle (minced)	+13	+14	with preliminary anaerobic period
	+15	+34	
	+33	+84	
Rabbit muscle (minced)	+3	−2	without anaerobic period
	−2	0	
	+6	+9	
Rabbit brain (minced)	+2	−1	
	+5	+4	

These experiments point out that the primary effect of thyroxin on cells consists in an accelerated splitting of protein and the split products so produced lead secondarily to an increase of combustion, similar to the effect of some amino-acids.[74]

That thyroxin displays its effect in the organism in a similar way is demonstrated by its metabolic action on frogs, in which many authors, including ourselves,[75] could not trace an effect on O_2-consumption. When, however, we analyzed the effect of thyroxin on protein metabolism in these animals, we found the interesting fact that of the two effects observed on the cells of warm-blooded animals, the furtherance of protein splitting and the increase of combustion, only one, namely protein splitting, takes place, whereas the oxidation effect is lacking. This is probably because the concentration of the breakdown products is not sufficient, or is reached too slowly in cold-blooded animals, to call forth the stimulating effect on oxidation processes.

Diagram 13 shows this effect of thyroxin on the N-excretion of frogs.

Our experiments on isolated cells leave no doubt that thyroxin influences directly the metabolic processes of the cells; they also explain why this immediate effect on the cells was lacking

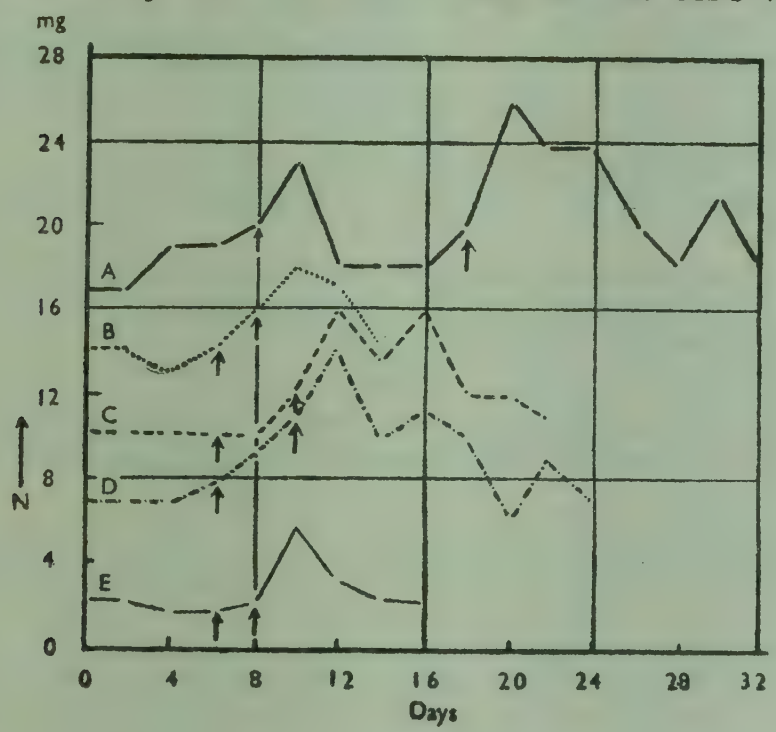


DIAGRAM 13.

Effect of thyroxin on N-excretion of frogs. (G. Mansfeld and A. Lánczos. *Arch. exp. Path. Pharm.* (1936), 183, 267.)
A. June–July 1935, weight of frog 80 gm.
B. May 1935, weight 78 gm.
C. July–August 1935, weight 62 gm.
D. September–October 1935, weight 80 gm.
E. March 1936, weight 80 gm.
At ↑ injection of 0.25 mg. thyroxin.

in most experiments. This brings us a step nearer to the solution of the question whether the thyroid hormone displays its effect in the organ cells or through stimulation of " metabolic centres ", a question of fundamental importance for the physiology and pathology of metabolism and heat production. The centralistic conception lost its conclusive force, because there is no doubt left that thyroxin is capable of accelerating combustion in the cell. Further experiments were necessary to prove whether this applies to the intact organism as well.

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CHAPTER VII

THE EFFECT OF THYROXIN ON SURVIVING ORGANS

THE first experiments proving that thyroxin, when taken up by the peripheral organ cells and administered to the whole animal, increases combustion in them, were made by Rohrer[76] and Dresel.[77] These workers demonstrated that the isolated kidney- or liver-cells of animals, which had previously been treated with thyroxin, show a higher O_2-consumption than corresponding organs in normal animals. To follow up these experiments, we examined whether the effect of thyroxin on the peripheral cells of one and the same animal can be proved by removing under strict asepsis, before and after administration of thyroxin, organs or organ fragments and analyzing them as to their O_2-consumption. (Mansfeld and Scheff-Pfeifer.[78])

These experiments did not only lead to a localization of the thyroxin effect in the organism, but also gave us a general method by which we succeeded in testing any changes occurring in the O_2-consumption of the various surviving organs *in vitro* before and after administration of medicines, poisons, hormones, and other treatments, i.e., heat, cold, oxygen deficiency, etc. For this purpose, we removed before and after treatment with various substances one parallel organ or, in single organs such as the liver, organ fragments, and after suspending them in pure O_2, without chopping them up, we were able to read their O_2-consumption manometrically. This method, which has served us splendidly for many years past, is described below.

(*a*) THE METHOD AND ITS EXAMINATION

The removed organs or fragments thereof are hung either in full, like muscles, or in large pieces of about 0.05–0.1 gm. in weight, in a Warburg vessel whose space is *filled with pure oxygen*; their oxygen consumption is measured manometrically, while the CO_2 formed, which diffuses from the organ, is absorbed by alkali. In order to be able to use the vessel unaltered, we usually apply a small hanging arrangement made of stainless steel, as can be seen in Diagram 14.

The muscle is tied up directly by its end (for instance with the Achilles' tendon) and hangs down freely between the two

posts. For other organs, such as the brain, liver, and kidney, small twisted baskets are used, which do not obstruct the access of oxygen. The organ to be examined is put into such a basket and is suspended in the apparatus. At the bottom of the vessel there is the usual quantity of Ringer's solution, osmotically equivalent to animal's serum, which ensures water vapour saturation. The volume of the hanging apparatus is established by its loss of weight in water. The organs can, of course, if necessary, be dried after use and weighed, and the O_2-values can be expressed in terms of mg. dry weight per hour.

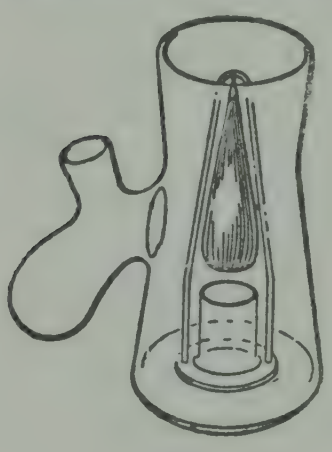

DIAGRAM 14.

The principle of the procedure to determine the O_2-consumption on whole organs is not new and was used by Thunberg[79] and Winterstein[80] for the spinal cord in frogs, by Parnas[81] for the muscle, and by Rohrer[76] for the kidney. Its abandonment is due to Warburg's[59]—theoretically correct—insistence that the slices in the investigation should be thin enough to ensure diffusion even at very low O_2-pressure and thus render the O_2-consumption independent of the O_2-tension. If it is a matter of determining the maximum respiration of cells, then Warburg's demand is justified; his results, however, do not give us any picture of the conditions prevailing in the organism, where the cells and their capillaries 2/3 closed in resting condition (Krogh[65]) are poorly supplied with O_2.

Whether this method can be put into practice for the above purposes depends on:

(1) whether in case of the application of pure oxygen as gas space, the O_2-supply of the organ cells is ensured, even in layers 2–7 mm. thick, as in gastrocnemius of frogs, guinea pigs and rabbits;

(2) whether the O_2-consumption of the undamaged organs shows a similar O_2-consumption as *in situ*;

(3) whether parallel organs or organ fragments removed after an interval of one or two days produce equivalent values.

1. *That the O_2-supply of undamaged muscles at* 100 *per cent* O_2 *is sufficient could be tested by making one of the parallel muscles breathe pure O_2 and the other O_2-N_2-mixtures of known composition.*

TABLE X

O_2-consumption of Muscle of warm-blooded Animals at Various Pressures of O_2.

Experiment No.	O_2%	Mm.³ O_2-consumption per gm. per hr.	Difference %
1	100	253·0	
	70	182·0	—28
2	100	268·1	
	70	197·4	—26
3	100	281·7	
	70	235·5	—16
4	100	215·7	
	80	174·3	—19
5	100	264·6	
	80	201·0	—24
6	100	241·3	
	80	207·9	—13
7	100	307·6	
	90	309·6	± 0
8	100	240·7	
	90	218·1	— 9
9	100	277·1	
	90	278·2	± 0
10	100	271·7	
	90	268·3	— 3
11	100	282·6	
	90	285·2	± 0
12	100	182·5	
	90	176·6	— 2
13	100	301·5	
	90	296·4	— 1·6
14	100	160·9	
	90	161·5	± 0
15	100	266·4	
	90	269·2	± 0

As we see, a maximum consumption of O_2 is reached in all the experiments at an O_2-concentration of 90 per cent, so that it is futile to assert that the muscles would have consumed more oxygen if they had more of it at their disposal as a result of better diffusion conditions. Their O_2-consumption is determined, as in the organism, by their requirements, and from 90 per cent O_2 onwards it is independent of the quantities offered.

A comparison between the O_2-consumption of intact brain and kidney fragments hung up in oxygen and that of similar organ sections suspended in Ringer's solution demonstrates that in our method the O_2-diffusion is sufficient for other organs as well. Table XI gives a survey of these experiments.

TABLE XI

Qo_2 Value—Rabbit Brain				Qo_2 Value—Rabbit Kidney			
Experiment No.	Slices suspended in Ringer's solution Warburg's method	Experiment No.	Rabbit brain in oxygen	Experiment No.	Slices suspended in Ringer's solution Warburg's method	Experiment No.	Rabbit kidney in oxygen
1	4·3 3·7	8	3·1 3·0	1	10·5	8	8·2
2	3·6 3·7	9	4·3 4·3	2	12·5	9	9·0
3	3·6 4·0	10	6·7 6·8	3	11·2	10	8·7
4	3·0 2·9	11	5·6 5·6	4	8·3	11	10·5
5	3·8 4·0	12	2·5 2·7	5	8·3	12	9·1
6	5·0 4·5	13	2·8 2·9	6	9·9	13	11·7
7	5·5 5·6	14	2·9 3·1	7	10·2	14	8·5
Mean	4·1		4·0		10·1		9·4

2. *In order to show that the values of O_2-consumption obtained in our procedure coincide with those observed in the organism we compare, in Table* XII, *these values with those in an isolated leg of a dog (Nils Alwall*[82]*) and a living cat (Verzar*[83]*), obtained through blood-gas determinations.*

TABLE XII

O_2-consumption in c.mm. per gm. per hour.

Undamaged frog gastrocnemius	Undamaged guinea-pig gastrocnemius	Artificially transfused surviving dog muscle	Gastrocnemius from living animal (cat) in Verzar's experiments
Warburg's apparatus 38°C.			
216	219	252	192
256	220	218	516
236	238	216	420
280	267	204	144
245	236	264	366
221	235	183	216
212	280	163	384
250	267	306	180
283	244	235	192
	240	190	402
	243	283	168
	258	270	174
	263	187	138
	243	227	
	246		
	249		
	250		
	223		
	235		
	241		
Mean 244	Mean 245	Mean 228	Mean 268

3. *The fact that organs removed after an interval of one to two days have an identical O_2-consumption is of paramount importance for our investigations; Table* XIII *demonstrates this for the muscles, while Table* XIV *proves it for the liver, kidneys, parotis, and testicle.*

TABLE XIII

O_2-consumption of the Left and Right Gastrocnemius of Guinea-pigs, taken at intervals of 24 hours.

Experiment No.	Gastrocnemius	Weight of muscle gm.	O_2-consumption mm.³ per gm. per hr.	Difference %
1	r	1·36	220·0	0·4
	l	1·44	219·0	
2	r	1·07	267·9	1·2
	l	1·01	238·7	
3	r	1·27	235·7	0·5
	l	1·32	236·9	
4	r	1·14	267·3	5·0
	l	1·81	280·6	
5	r	1·28	240·6	1·4
	l	1·21	244·2	
6	r	1·23	258·1	5·9
	l	1·38	243·6	
7	r	1·33	243·5	8·1
	l	1·26	263·2	
8	r	1·12	249·6	1·1
	l	1·10	246·9	
9	r	1·27	249·9	11·6
	l	1·32	223·8	
10	r	1·28	241·7	2·2
	l	1·18	236·5	

TABLE XIV

O_2-consumption of Isolated Organs of Rabbits, taken at intervals of 24–48 hours.

Experiment No.	Date	Mm.³ O_2-consumption per gm. per hr.		Difference %	Interval hrs.
		1	2		
	LIVER				
9	5 II 1938	530·0	471·0	—11	48
10	26 I 1938	674·3	619·9	— 8	24
11	15 XII 1937	479·0	423·0	—11	24
12	20 XII 1937	529·5	532·0	+ 0·4	24
13	22 VI 1938	593·5	554·5	— 6·5	24
14	23 VI 1938	623·9	651·8	+ 4·6	24
15	24 VI 1938	669·7	687·0	+ 2·5	24
	KIDNEY				
16	30 VI 1938	1103·5	1006·4	— 8·2	24
17	8 VII 1938	1091·7	1083·4	— 0·7	24
18	13 VII 1938	788·4	780·5	— 1·0	24
	PAROTIS				
19	24 I 1939	417·0	412·2	— 1·0	48
20	24 I 1939	397·4	355·9	— 1·0	48
	TESTICLE				
21	24 I 1939	333·2	354·4	+ 6	48
22	24 I 1939	196·5	180·6	— 8	48

From these investigations it follows that this method is most suitable for finding out whether certain substances which alter the rate of oxygen consumption, like thyroxin for instance, accelerate directly the oxidation system of the organ cells or whether the alteration of the metabolism is conditioned by other influences like the central-nervous impulses. This method is of particular service when we seek information regarding oxidations

in the inactive muscle cell. Until now only diaphragm could be utilized in Warburg's experiment, a point specially stressed by Warburg himself, because sliced muscles do not give any physiological values.

Verzar[83] showed that combustion in the muscle is a function of its blood supply and it seems probable that the combustion can be increased also in the muscle at rest through central-nervous impulses (the so-called chemical muscle tonus). When a substance which stimulates oxidation gets into the organism, it is difficult to decide for certain whether the altered combustion of the organs *in situ* is conditioned by a direct effect on the cell oxidation, by influences of the vessel or by central-nervous impulses. The investigations of v. Issekutz, jun., and Harangozo-Oroszy[83a], prove it amply; in these the effect of α-dinitrophenol was determined by blood-gas analysis in the hind legs of dogs, whilst in cut muscle pieces of the same hind legs it was determined according to our method. A powerful increase of the flow of blood occurred in the hind legs, as the authors proved with Rein's Stromuhr, and as the animal had 40°C. high fever, probably also an increase of combustion conditioned centrally by tremor of the muscles. *Correspondingly, the O_2-consumption in the* in situ *musculature was substantially higher (increase up to* 890 *per cent) than in the cut-out muscle, where, obviously, only the direct effect of dinitrophenol on the muscle cell was noticeable without other disturbing influences. Our method aims at finding out this effect.*

(*b*) Localization of the Thyroxin Effect in the Body

This method allowed a systematic examination of the question whether increased combustion in the organ cells, caused through thyroxin, also continues after their separation from the nervous system, i.e., is peripherally conditioned, and in which organs the increase occurs.

Our experiments answered both these questions in such a way as to be of decisive importance for further progress.

They demonstrated that in the organs of one and the same animal (as Rohrer[76] and Dresel[77] saw it on control animals) the thyroxin effect on the combustion continues, when the organs are separated from the body, i.e., also from the central nervous system.

In Table XV we see the result of such experiments.

It was demonstrated that, in all examined *vegetative* organs, thyroxin brings about an increase of O_2-consumption, which continues also after isolation of the organs. Hence it follows that the increase of combustion in the organs, caused through thyroxin, is not a result of an irritation of metabolic centres but of a peripheral

TABLE XV

O_2-consumption of Isolated Organs of Rabbits before and 24–48 hours after Subcutaneous Injection of 1 mg. Thyroxin.

Experiment No.	Date	O_2-consumption in mm.³ per gm. per hour		Difference %	Maximum Increase in	Interval in hours
		before Thyroxin	after Thyroxin			
	LIVER					
23	21 XII 1937	400	524	+31	1st hour	24
24	21 XII 1937	406	634	+57	1st hour	24
25	22 XII 1937	527	517	± 0	—	24
26	13 I 1938	285	394*	+38	1st hour	48
27	13 I 1938	365	460*	+26	1st hour	48
28	15 I 1938	271	379*	+39	1st hour	24
29	15 I 1938	344	515*	+48	1st hour	24
30	24 I 1938	507	625	+23	1st hour	24
	PAROTIS					
31	20 II 1939	301	336	+11	1st hour	24
32	20 II 1939	270	331	+22	2nd hour	24
33	20 II 1939	209	361	+72	1st hour	24
34	17 I 1939	195	315	+61	2nd hour	24
	TESTICLE					
35	30 I 1939	270	263	± 0	—	48
36	30 I 1939	200	275	+37	1st hour	48
37	7 II 1939	251	293	+56	1st hour	48
38	7 II 1939	195	308	+58	1st hour	48
	KIDNEY					
39	9 III 1939	727	854	+17	1st hour	48
40	9 III 1939	654	797	+21	1st hour	48

* 0·2 mg. Thyroxin injected subcutaneously

influence. A constant effect of increased innervation on combustion after cessation of the nerve stimulation is unknown in physiology, nor was it ever observed in experiments made with this end in view. (Mansfeld.[84])

In regard to the question of *which are the organs* where increased combustion takes place after supply of thyroxin, the experiments had an interesting result. They showed that the increase of the basal metabolism is not performed by the musculature, as one whould have expected, but by the vegetative organs.

TABLE XVI

O_2-consumption of Isolated Gastrocnemius before and after Subcutaneous Injection of Thyroxin.

Experiment No.	Date	O_2-consumption in mm.³ per gm. per hour		Difference %	Interval in hours	Animal
		before Thyroxin	after Thyroxin			
40	11 XI 1937	162·6	159·2	—20	24	Rabbit
41	11 XI 1937	151·5	174·2	+15	24	
42	20 XI 1937	182·9	186·7	+ 2·2	48	
43	7 III 1939	135·0	141·0	+ 4·4	24	
44	7 III 1939	195·0	184·5	— 5·0	24	
45	2 XII 1937	265·8	257·0	— 3·0	24	Guinea-pig
46	2 XII 1937	225·0	251·0	+11	24	
47	2 XII 1937	218·0	222·0	+ 1·8	24	
48	4 XII 1937	257·5	243·0	— 5·4	48	
49	4 XII 1937	259·0	266·0	+ 2·7	48	

On the skeletal muscle, namely on the gastrocnemius, an increase of combustion could never be demonstrated (see Table XVI) not even after an interval of three days from the intake of thyroxin.

This is remarkable because it is impossible to say that the gastrocnemius is not suitable for such experiments. As a survey of Table XVII shows, combustion in the gastrocnemius is substantially increased after treatment with dinitrophenol.

TABLE XVII

O_2-consumption of Gastrocnemius of Rabbit before and after Subcutaneous Injection of 15 mg./kg. Dinitrophenol.

Experiment No.	Gastrocnemius before and after dinitrophenol	Weight of muscle gm.	O_2-consumption mm.³ per gm. 1st hour	Increase %	O_2-consumption mm.³ per gm. 2nd hour	Increase %
1	before	1·46	166·6	79	174·2	46
	after	1·36	299·0		255·0	
2	before	1·50	176·3	55	174·4	42
	after	1·50	174·8		249·0	
3	before	1·46	177·2	57	166·4	61
	after	1·06	279·0		272·0	
4	before	1·46	154·0	94	161·0	63
	after	1·36	300·0		263·2	

In Chapter X we shall see that the isolated gastrocnemius is particularly suitable for determining substances which increase combustion.

Contrary to the vegetative organs, striped muscle shows no increase of combustion after treatment with thyroxin. A similar result was obtained in the experiments made in my institute by Nils Alwall and Irene Scheff-Pfeifer.[82] The synergistic action of thyroxin and α-dinitrophenol, described for the first time by Alwall[85] was to be tested on one leg of a dog. For this purpose, the hind legs of dogs, of which some were normal and some treated with thyroxin, were perfused with defibrinated dog's blood, and their O_2-consumption was determined before and after the addition of α-dinitrophenol and later also of methylene blue.

The procedure elaborated for this purpose is the following: *

The apparatus differs from the usual one in that the perfused leg is neither separated from the animal nor is it tied off with a ligature. The leg remains *in situ* and loss of blood is prevented by a ligature of the vessels, so that the transfusion takes place under completely physiological conditions. One proceeds as follows:

The animal is tied up without morphine, the tracheal cannula tied off in novocain-anaesthetic, and the animal is heavily narcotized by blowing in ether (Starling pump) through artificial respiration. The abdomen is opened and bleeding is stopped by ligatures. Then the viscera are removed with the exception of the kidneys and liver, and bleeding is prevented by strong ligatures.

* During the elaboration of this method I was pleased to have the assistance of Dr. Robert v. Wertz of Cologne.

The abdominal aorta is then freed for a length of 6–8 cm. above the dividing point, and, starting with the kidney vessels, all branches up to the division as well as the joint stem of the hypogastric artery are tied off. Now the cannula of the arteries is tied off as near as possible to the dividing point, whereas the cannula of the veins is tied off in the vena cava as high up as possible in order to contain the whole of the venous blood. If it is desired to transfuse only one leg, this is done by unilateral ligature of the iliac artery and vein. If both legs are to be irrigated *separately*, the cannulae are tied off with the iliac artery and vein.

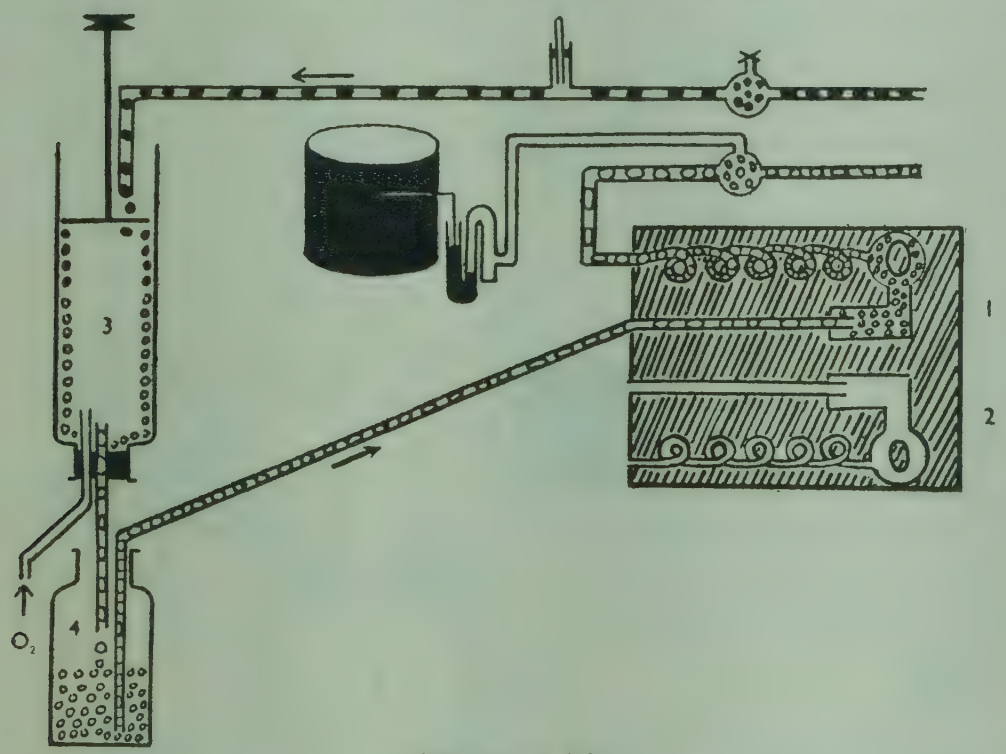

DIAGRAM 15A.

Scheme of transfusion of isolated dog's leg. Determination of gas-exchange by blood-gas analysis.

1, 2. Dale-Schuster pumps.
3. Artificial lung with revolving disc.
4. Container for arterial blood.
o o o Arterial blood.
• • • Venous blood.

After the cannulae are tied off, transfusion is commenced as soon as possible. The time during which the hind legs remain bloodless amounts to 2–3 minutes. The transfusion is done with defibrinated canine blood by means of one or, in the case of separate transfusion of the legs, two Dale-Schuster pumps. (See Diagram 15A, 1 and 2.)

In order to create the best possible physiological conditions, the blood is fortified with 3 gm. dextrose and 10 units insulin per 1½ litres (Ruehl[86]). The temperature of the outflowing blood is constantly measured and kept at 37°C. by warming the

arterial blood and the leg. The outflowing venous blood, the stream speed of which can be measured continuously, enters one of two "artificial lungs" with a rotating disc. (Diagram 15A 3.) After saturation with O_2, the blood reaches a common or two separate containers according to need, from where it is pumped again into the legs. The rate of transfusion in our experiments was 90–120 cc. per minute. Blood removal for the oxygen determination took place simultaneously from the incoming and outgoing blood, and the blood was caught under paraffin. Directly before and after every blood removal, the irrigation speed was established.

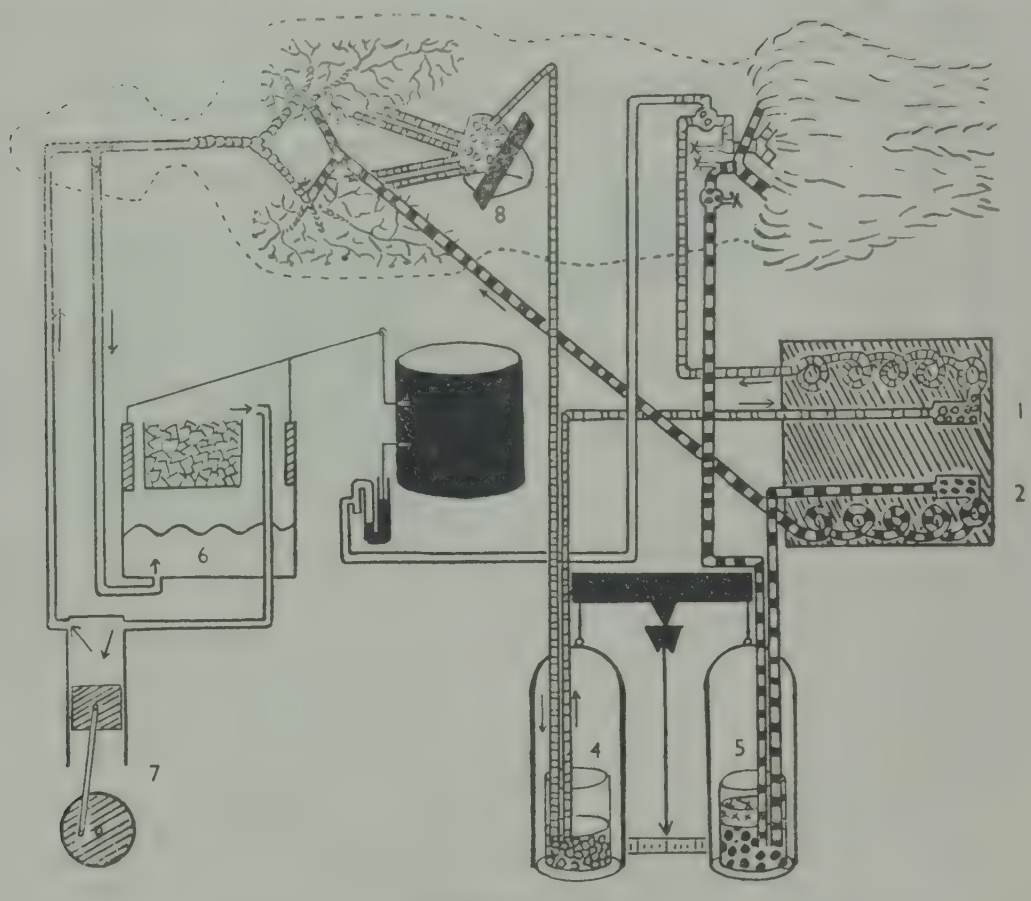

DIAGRAM 15B.

Scheme of transfusion of isolated dog's leg. Graphical registration of gas-exchange.

1, 2. Dale-Schuster pumps.
4. Container for arterial blood.
5. Container for venous blood.
6. Apparatus for graphical registration of gas-exchange.
7. Starling pump for artificial respiration.
8. Clamp for the ventricles.
o o o Arterial blood.
• • • Venous blood.
x x x Liquid paraffin.

The experimental apparatus is shown in diagram 15A.

The determination of the O_2-consumption was done by colorimetric determination of the O_2-saturation of arterial and venous blood according to the splendid but little known method of Hollo

and Weiss.[87] Our comparative measurements of oxygen consumption after this method and that of Van-Slyke[88] coincided well. For instance: the O_2-consumption in an experiment was per kg. leg per minute (0°C., 760 mm. Hg) before addition of dinitrophenol, 4.4 c.c. (after Hollo and Weiss), 4.2 c.c. (after Van-Slyke); after addition of dinitrophenol 8.1 c.c. (after Hollo and Weiss), or 8.3 c.c. (after Van-Slyke).

Recently we altered this procedure so that the determination of the gas change need no longer be done by intermittent blood-gas analysis, but is constantly registered graphically. When we wish to determine the O_2-consumption alone by means of Krogh's small apparatus, and to obtain the CO_2-production as well, we use a respiration apparatus of our own construction[89] adapted to the size of the experimental object. The procedure is schematically demonstrated in Diagram 15B.

We proceed as follows:

First, the transfusion of the legs is done as described above, the arterialization of the blood occurs through an artificial lung. (Diagram 15A 3.) As soon as the transfusion has begun the thorax of the living animal is opened, a cannula filled with defibrinated canine blood is tied up in the pulmonary artery, and the lungs are transfused by means of the second Dale-Schuster-pump. The heart ventricles are clamped off with a large stomach clamp. The blood flows through a cannula tied up in the left auricle into the arterial container (4), whence it is despatched into the abdominal

TABLE XVIII

O_2-consumption of Isolated Hind Legs of Dogs, with Normal and with Preliminary Thyroxin Treatment.

O_2-consumption : mm.³ per kg. per min.	
Normal	Preliminary Thyroxin treatment
6·2	4·5
3·9	4·7
4·6	5·7
5·7	5·4
5·3	3·1
3·4	3·7
4·4	—
8·2	4·5
4·6	—
Mean 5·1	Mean 4·5

aorta through pump (1). The venous blood from the legs flows into the venous container (5) where it is protected from oxygen in the air by a layer of liquid paraffin. The venous blood reaches the lungs through pump (2). These are made to breathe artificially by means of a Starling pump (7) via the metabolic apparatus (6). The containers for the arterial and venous blood stand on a balance so that

any inequality of both circular courses is noticed immediately and can be corrected by adjusting the output of the pumps.

The values given below have been obtained through the first method, i.e., through blood-gas analysis.

It was demonstrated that the isolated muscles of dogs which have had preliminary thyroxin treatment did not consume more O_2 than the muscles of normal animals. (See Table XVIII). This is similar to the gastrocnemii of rabbits and guinea pigs.

The experiments also showed that thyroxin penetrates the muscle cells but that its effect is latent until an oxidation stimulant in the muscle cells increases combustion; this stimulus becomes stronger than usual as a result of the presence of thyroxin.

TABLE XIX

Percentage Increase of O_2-consumption using Dinitrophenol and Methylene Blue.

Substance added to transfused blood	Normal	Preliminary Thyroxin treatment
Dinitrophenol	73 118 98 124	220 170 176
Methylene Blue	102 71 116 149 120	227 249 208 237
Mean	108	212

When dinitrophenol or methylene blue was added to the transfused blood, an increase of oxidation of about 100 per cent could be noticed on the muscles of animals which received preliminary thyroxin treatment. (See Table XIX.)

An increase in the temperature of the surroundings also provokes the latent thyroxin effect; this was observed on the gastrocnemii of frogs, which were cut out before and after thyroxin administration and examined as to their O_2-consumption in a series of experiments at 20° C. and at 38° C. It was demonstrated, as Table XX shows, that while at 20° C. the muscles before and after thyroxin treatment give the same values with one exception, at 38° C., a temperature unnaturally high for the frog's muscle, we saw an increase of combustion after thyroxin treatment.

The experiments demonstrated the remarkable fact that thyroxin does not accelerate gas change in the muscle at rest as in the vegetative organs, but only creates in it the conditions under which oxidation stimuli like dinitrophenol, methylene blue—or in a cold-blooded animal, increase of temperature—are made more

effective, whereas a thyroxin increase of basal metabolism is accomplished by the vegetative organs.

The discovery of Abderhalden and Wertheimer,[90] that glycogen is already substantially lowered in a constantly active heart under influence of thyroxin while it is still unchanged in a resting muscle, coincides well with the above findings. The work of Heinrich Schumann,[91] according to which the almost ineffective thyroxin quantities at rest lead, in case of increased activity of the heart, to a substantial reduction of phosphagen, glycogen, and adenyl-

TABLE XX

O_2-consumption of both Frogs' Gastrocnemii before and 48 hours after Injection of 0.5 mg. Thyroxin.

Experiment No.	Date	O_2-consumption mm.³ per gm. per hour		Increase %	Temperature °C
		before Thyroxin	after Thyroxin		
62	1 II 1939	64·2	67·4	4	20
63	1 II 1939	63·8	65·4	0	
64	1 II 1939	60·1	69·6	15	
65	1 II 1939	63·4	70·0	9	
66	1 II 1939	75·6	78·4	3	
67	4 II 1939	73·1	72·3	0	
68	4 II 1939	58·9	60·2	0	
69	6 II 1939	52·0	50·4	0	
70	6 II 1939	60·0	56·7	0	
50	9 IV 1937	207·2	242·6	17·5	38
51	10 IV 1937	235·4	270·2	15	
52	10 IX 1937	197·6	242·6	22	
53	28 I 1939	150·0	182·2	21	
54	28 I 1939	159·4	213·6	34	
55	28 I 1939	158·2	180·3	14	
56	28 I 1939	132·5	131·7	0	
57	11 II 1939	132·3	171·6	30	
58	11 II 1939	164·2	180·1	10	
59	11 II 1939	179·5	164·5	0	
60	11 II 1939	158·6	202·1	27	
61	11 II 1939	150·3	177·2	18	

pyrophosphate, proved the latent thyroxin effect, which becomes apparent through oxidation stimuli, including, of course, increased activity. There are interesting experiments of Chachovitsch[92] in which he measured, after thyroidectomy, not only the basal metabolism but also the so-called peak-metabolism in an extremely cold temperature. It could be shown that after a certain time the basal metabolism in a thyroidectomized animal is hardly diminished any further, but that the maximum combustion in cold temperatures shows greatly reduced values in comparison with the period before thyroidectomy.

We have recently found the interesting fact that the tonus muscles of the neck, contrary to the leg musculature, increase their oxidation under the influence of thyroxin as shown by Table XXI.

All these experiments show that thyroxin, after its incorporation into the body, gets into the peripheral organs and either

furthers directly the combustion in them or creates conditions, as in the resting skeletal muscle, due to which oxidation stimuli act more strongly than otherwise. The next question was: Does this hormone *in addition to its peripheral effect* also increase the basal metabolism by stimulation of the so-called metabolic centres? This would be of importance for the physiology and pathology of metabolism.

TABLE XXI

O_2-consumption of Isolated Neck Muscle (sternocleidomastoid) before and after Injection of Thyroxin.

Date	O_2-consumption in mm.³ per gm. per hour		Difference %
	before Thyroxin	after Thyroxin	
8 IV 1941	256	288	+12·5
8 IV 1941	242	290	+19·7
12 IV 1941	378	465	+23·0
12 IV 1941	362	452	+25·0
16 IV 1941	306	320	+ 4·0
16 IV 1941	288	357	+24·0
22 IV 1941	311	325	+ 4·5
22 IV 1941	291	433	+49·0
24 IV 1941	249	279	+12·0
24 IV 1941	233	287	+23·0

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CHAPTER VIII

IS THERE A CENTRAL-NERVOUS EFFECT OF THYROXIN ON BASAL METABOLISM?

THE experiments reported on the effect of thyroxin on cells and cell groups brought us an essential step forward in the question as to whether the metabolic effect of thyroxin attacks peripherally or centrally. The centralistic conception lost most of its conclusive force, for nobody doubted any longer that thyroxin was capable of accelerating combustion directly in the cell. The question, however, whether thyroxin increased combustion further by stimulation of the so-called metabolic centres remained still unsolved, and many an observation seemed to strengthen this possibility.

If we deal with this question in detail, we do it not only because it is desirable to clarify the point of attack of so important a hormone as thyroxin, but also because it is a matter of a fundamentally important principle. We find, particularly in the clinical literature, in ever-increasing numbers, the opinion that the so-called metabolic centres, which are situated in the mid-brain, increase combustion in the organ cells through direct *nervous impulses*. The idea that there exists a general " chemical organ tonus " similar to the so-called " chemical muscle tonus, " and that the cells of all organs receive combustion-increasing impulses via the autonomic nervous system is, as we shall see, without foundation, because *these metabolic centres command, more probably, the endocrine glands,* the products of which—amongst them thyroxin in particular—increase the combustion in the cells. The strongest support for the theory of central-nervous increase in metabolism is now considered to be the metabolic effect of thyroxin; but the belief that this is centrally conditioned is based on false observations and experimental errors.

If we examine the question whether there really is a central-nervous increase of basal metabolism by thyroxin, on the basis of the assembled facts and material, the central problem is the increase of *basal* metabolism. It is not denied in any way that besides this physiological thyroxin effect there is a pathological or toxic one also, where central nervous excitations dominate. That both these effects of thyroxin, *increase of basal metabolism* and *increased organ function,* have to be separated was already known when the first metabolic experiments on patients suffering from Graves' disease were made at Naunyn's clinic, and it was demon-

strated that after elimination of the central irritations—namely of the tremor through hyoscin—there remains a considerable increase in basal metabolism. (Magnus-Levy.[93]) That thyroxin possesses two effects entirely different in nature was demonstrated by Josef Sos[94] on rabbits, in which, by means of the apparatus described by Kochmann and Kuntz,[95] the impetus to movement and the gaseous exchange while at rest were measured simultaneously, before and after thyroxin administration.

The experiments demonstrated that the first effect of thyroxin is at least to double the *motility of the animals.* In one experiment *the increased motility was sixfold.* It disappears, however, after the first five hours. During this period the basal metabolism remains constant. An increase of combustion sets in, as known, only after 24 hours, reaches its culminating point after 48 hours, *during which time the motility of animals is fully normal.* Both effects of thyroxin, the irritation of the central nervous system characterized by motoric unrest and the increase of combustion, are quite independent and separated from one another temporally.

In harmony with this are experiments which also speak in favour of the peripheral point of attack of the metabolic effect. Thus, Riml and Wolff,[96] as well as Oberdisse and Roda,[97] demonstrated that the metabolic effect of thyroxin is not diminished after cutting the nerves. Also Aub, Bright, and Uridill[98] saw in hyperthyroid cats, after intersection of the plexus ischiadicus and brachialis, either a very small diminishment of combustion or mostly none at all.

Apart from some clinical observations made by Falta and Hoegler,[99] by Jehle,[100] and Leschke,[101] according to which people suffering from diseases of the brain, epileptic children, and patients with brain lues, behave refractorily with regard to thyroxin, there were three groups of experiments, in particular, which seemed to favour the view that thyroxin increases the combustion with a central point of attack: (1) the experiments of Issekutz and collaborators,[102] who sought to prove that thyroxin increases combustion through irritation of the heat centre, i.e., becomes apparent only where such a centre is available; (2) the experiments of Glaubach and Pick[103] on the antagonism novocaine-thyroxin, which is prevented through intersection of the spinal cord and must, therefore, be centrally conditioned, and (3) the work of Lina Stern[104] and colleagues,[105] who, after thyroxin injections into the side-ventricles, saw a powerful increase in basal metabolism after only a few hours and explained it, wrongly, as we shall see later, by a direct effect of thyroxin on the " metabolic centres ".

We shall analyze these experiments separately.

(*a*) THE ALLEGED EFFECT OF THYROXIN ON THE HEAT CENTRE

As far as the experiments of Issekutz are concerned, the hypothesis—that the metabolic effect of thyroxin is linked up with the existence of heat regulation—proved to be erroneous. Anna Lanczos and I[106] could prove a powerful increase of protein combustion in frogs, and Novak,[107] a pupil of Issekutz, proved such an increase for the O_2-consumption. This harmonizes with older observations of Abelin and Scheinfinkel,[108] as well as of Nagel,[109] who were able to prove an increase of the respiratory metabolism, the latter up to 40 per cent on cold-blooded animals.

In this way the basis of Issekutz's experiments was shattered.

DIAGRAM 16A

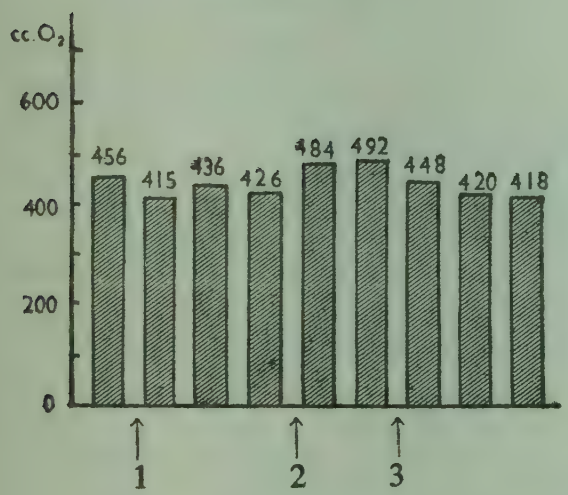


DIAGRAM 16B

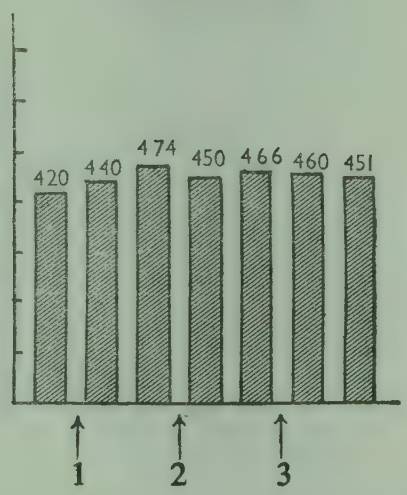


DIAGRAM 16A

Date, 23rd May, 1935. Weight of dog, 11.6 kg. Tracheal cannula inserted in local anaesthesia (Novocaine-Adrenalin). Artificial respiration by Starling pump connected air-tight to Krogh's apparatus. The dog lies free, completely motionless.

1. 0.1 gm./kg. Luminal-Sodium subcutaneously.
2. 0.5 gm./kg. Urethane intravenously.
3. Section of spinal cord at C 5. Blood pressure in mm. Hg: before section, 124; after section, 120.

DIAGRAM 16B

Date, 10th May, 1935. Weight of dog, 10.7 kg. Conditions as under 16A.

1. See Diagram 16A.
2. See Diagram 16A.
3. Section of spinal cord at C 5. Blood pressure in mm. Hg: before section, 124; after section, 96.

His theory of a central thyroxin effect on combustion was based mainly on two groups of experiments.[110]

(1) It was demonstrated partly on curarized, partly on non-curarized, animals that the O_2-consumption, which was increased

through thyroxin administration, becomes normal if the spinal cord of the animals is intersected in the cervical region.

(2) The thyroxin-conditioned increase of combustion disappears if we paralyse the heat regulation of the animals by administration of high dosages of luminal.

As far as cervical intersections of spinal cord on non-curarized animals are concerned, an increase of combustion in Issekutz's experiments was observed within a few hours after administration of very high dosages of thyroxin (up to 3 mg. per kg. body weight). This points not to on increase in basal metabolism, which sets in,

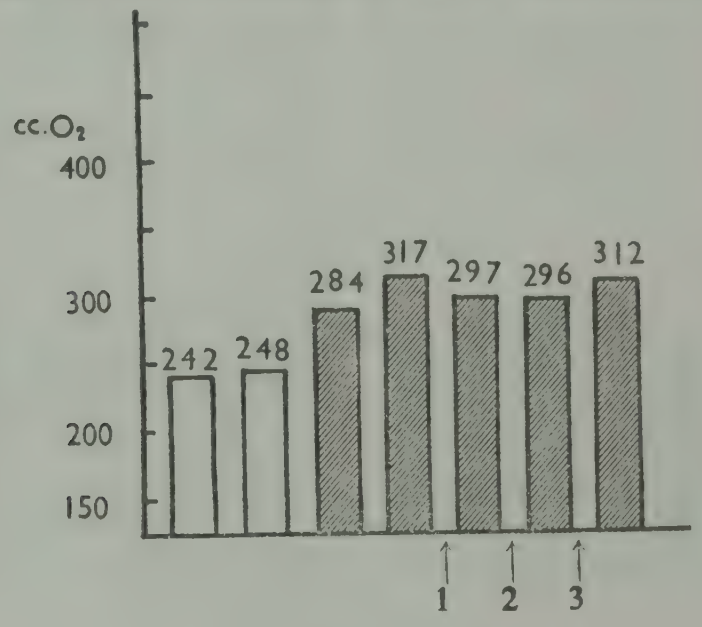


DIAGRAM 17

Weight of dog 6.7 kg. On 24th May, 1935, thyroidectomy, preserving two parathyroids, and tracheotomy for the insertion of a permanent cannula. This is replaced before each gas-exchange experiment by a buffer cannula connected through Starling pump with Krogh's apparatus. Before each metabolic experiment: 20 hours starvation. After the second and third metabolic experiments the dog received each time 50 gm. raw thyroid perorally. The real experiment (last four columns) was carried out on 31st May, 1935.

□ Normal.

/// After feeding of thyroid.

1. 0.1 gm./kg. Luminal.
2. 0.4 gm./kg. Urethane.
3. Section of spinal cord at C 5. Blood pressure in mm. Hg: before section, 138; after section, 134.

as is known, only after 24 hours, but to an increase in O_2-consumption conditioned by unrest and tremor of the muscles. This consumption naturally stops after intersection of the spinal marrow, or does not appear at all if thyroxin is administered to animals whose spinal cord has already been intersected. However, if we repeat the experiments, gradually raising the metabolism of animals through administration of thyroid and thyroxin so that, apart from the first few hours after the thyroxin intake, no motoric

unrest occurs, then cervical intersection of the spinal cord never leads to a decrease of the O_2-consumption. Intersection of the spinal cord of normal animals in which combustion also stays under thyroxin influence would have dropped after cutting the spinal cord, if it had been centrally conditioned. These experiments can be seen in Diagrams 16, 17, and 18.

We see that not even once was the O_2-consumption of our dogs, which was increased through thyroxin, diminished through intersections of spinal cord. Our experiments on the metabolism in white rats, in which we administered thyroxin to the animals

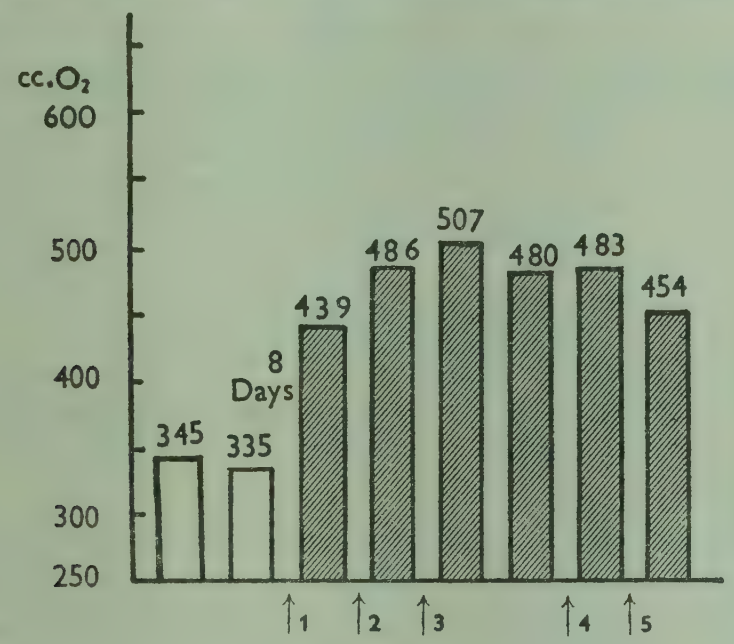


DIAGRAM 18

Weight of dog 10.5 kg. On 11th June, 1935, tracheotomy for insertion of permanent cannula. Metabolic experiment begun on 15th June, 1935, as in case of Diagram 17. The real experiment (last three columns) was carried out on 29th June, 1935.

□ Normal.

/// After feeding of thyroid.

1. Fed for 8 days with a total of 240 gm. fresh thyroid.
2-3. Receiving for 3 days a daily quantity of 50 gm. fresh thyroid.
4. 0.1 gm./kg. Luminal-Na and 0.5 gm./kg. Urethane.
5. Section of spinal cord at C 5.

intersected at C 6–C 7 and determined their gas change continuously,* led to the same result. Table XXII shows the result of three experiments on normal rats and three other rats whose spinal cord had been intersected.

The experiments show that the increase of combustion due to thyroxin on an animal whose spinal cord had been intersected occurs at the same time and to the same extent as on a normal one.

* For details of the experimental arrangement see Mansfeld, v. Tyukody, and Scheff-Pfeifer: *Arch. exp. Path. Pharm.* (1936), 181, 376.

The result of our experiments was confirmed by Issekutz and collaborators,[111] but, in spite of this, the theory of a central metabolic effect of thyroxin was not abandoned by them, because Issekutz believed that after intersection of the spinal cord, " there still remain numerous nerve fibres in the vagus and in the sympathicus which play an important role in the regulation of temperature and metabolism ". He repeated our experiments on thyroxin-treated cats in which he either eliminated the brain functions with large dosages of luminal, which paralyse even the deepest brain centres, or interrupted all connections between brain and

TABLE XXII

Increase of O_2-consumption on Animals, 24–26 hours after administration of Thyroxin.

Normal %	Intersected spinal cord %
31	19·7
12·2	13·3
23	25·4

periphery through resection of the medulla and the cervical vagi. *In order to maintain a normal blood pressure, a constant infusion of adrenalin was given.*

A most remarkable circumstance was that the animals received curare before the " brain elimination ", so that the O_2-consumption increased by thyroxin was in fact due to increased basal metabolism; a few hours after the " brain elimination " a decrease of the O_2-consumption recurred down to the norm and even lower.

These experiments seemed to us to be of the greatest importance, because, if their results were correct, the old issue whether there was a centrally conditioned increase of combustion in a muscle completely at rest would have been solved in a positive sense. It would also have been established that it was thyroxin which brings about this centrally conditioned increase of combustion and thereby attacks chemical heat regulation.

Not only the importance of this conclusion, but also a series of inconsistencies into which I cannot enter now, caused us to verify these experiments and to investigate whether these interesting results had not been conditioned in any way by some experimental errors.

First let us examine the experiments where the brain function was eliminated through large dosages of luminal. A weak point of these experiments was *the possibility that high dosages of luminal not only paralysed the brain centres but possibly reduced combustion in the periphery as well.* In order to eliminate this possible error, we used Heymans's[112] method of crossed-brain circulation.

We show the details of our experimental arrangement in Diagram 19.

1. *Preparation of Dog A for the Metabolic Experiment*

After fixing a constant tracheal cannula the basal metabolism of an unrestrained and fasting dog was determined daily by the method of Krogh, until the dog became accustomed to the method, and one obtained concurring values. Restless animals were given small quantities of luminal (0.03–0.05 gm. per kg.) before the metabolic test which, as we have just shown, did not diminish combustion. (See Diagrams 16, 17, and 18.) Then, for about a fortnight,

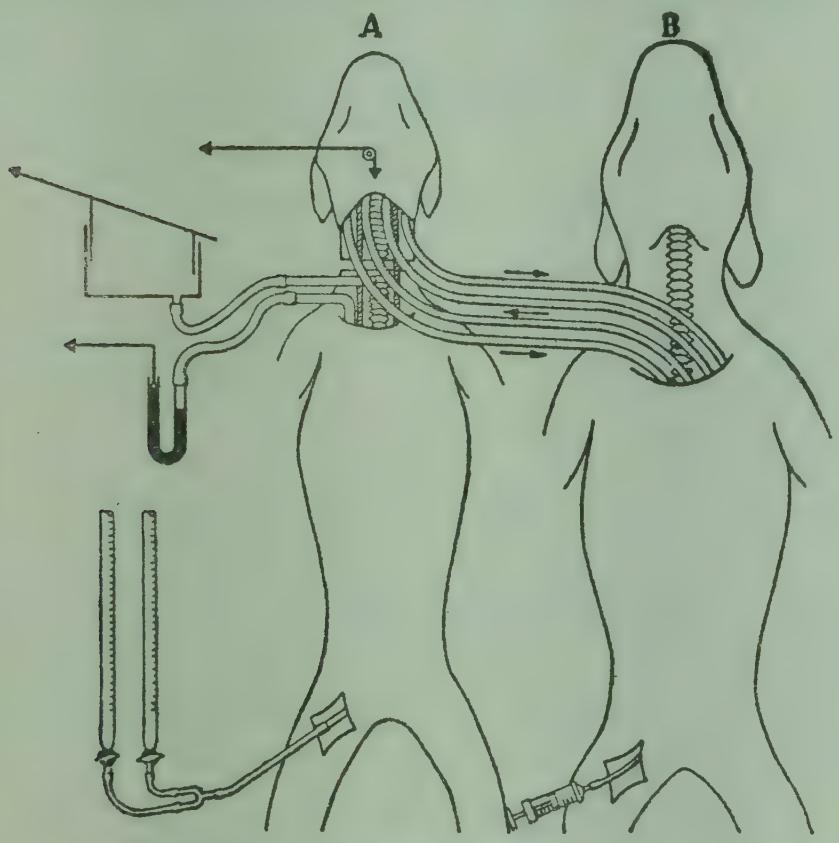


DIAGRAM 19

the metabolism was increased, partly through thyroxin injections, partly through feeding of raw thyroid. When increased basal metabolism was reached and established in at least three concurring experiments, we proceeded with the main experiment.

2. *The Main Experiment*

A second dog, Dog B, about one-third bigger than Dog A, was chosen and tied up. Under local anaesthesia the carotids and jugular veins were laid open in both dogs and connected with Payr's cannulae in such a way that Dog B, as blood donor, had to supply the head of Dog A. Now Chassaignac's ecraseur was fixed to Dog A at a height of between C 6–C 7 and throttled without touching the nerves which run along the throat. The animal has artificial breathing and the crossed brain circula-

tion was opened through removal of the clamps. Now Dog A was poisoned with curare via the femoral vein until the patellar reflex disappeared, which was a good indicator of motoric nerve paralysis. As the curare reached only the body of the animal, the head muscles remained active, and the very lively, sometimes stormy, reactions, as well as regular head breathing and blood pressure of a normal level in Dog A's body, indicated the intact functioning of the brain centres.

The blood pressure of Dog A was registered continuously from the central carotid stump. The metabolic experiments began by connecting the Starling pump with our Krogh apparatus for dogs.

TABLE XXIII

Dogs with crossed-brain circulation.

Experiment No.	Before cross-circulation		Before injection of luminal		After administration of luminal								
	V norm. W Date	V after Thyroxin W Date	V after curare W Date	θ P	T γ	θ P	O_2	T γ	θ P	O_2	T γ	θ P	O_2
1	3480cc 11·0 12 VII	5028cc 10·5 18 VII	4560cc 9·6 20 VII	38·5°C 90	5′ 0*	38·5°C 94	4512	30′ 0*	38·6°C 84	4188 (–8%)	1h 5′ 1·0	38·2°C 94	4488 (–1.7 %)
2		4830cc 8·3 25 VII	4584cc 8·1 26 VII	38·8°C 90	10′ 0*	39·0°C 70	4488 (–2%)						
3	3900cc 9·3 24 IX	5304cc 8·5 9 X	5160cc 8·2 10 X	39°C 60	1h 20′ 0·2	38·8°C 60	4908	2h 20′ 0·2	38·7°C 76	4836 (–6%)			
4	4440cc 13 25 IX	6336cc 11 3 X	5892cc 10 8 X	39·4°C 64	2h 40′ 0·28	39·0°C 68	5532 (–6%)						
5	4836cc 12·4 10 X	6504cc 11·5 26 X	6204cc 11·4 27 X	39·2°C 70	30′ 0·3	39·3°C 70	6168 (±0%)	1h 25′ 0·3	39·4°C 70	6168 (±0%)			

V = O_2-consumption in cc. per hour.
P = arterial blood pressure in mm. Hg.
γ = infused quantity of adrenation in γ per kg. per hour.
T = time after administration of luminal in hours and minutes
θ = body temperature in °C.
W = weight of animal in kg.
* Blood pressure sustained with saline infusion.

Having thus determined the O_2-consumption in at least four 6-minute periods in the course of 1–1½ hours, the blood donor, Dog B, received injection of 0.15 g. luminal per kg. into the femoral vein, slowly, during 15–20 minutes, and, if necessary, artificial respiration was introduced. A complete paralysis of the brain of Dog A took place during the injection, manifested through cessation of head breathing, extinction of all reflexes as well as paralysis of the vasmotoric centres. (Dropping of the blood pressure of Dog A.) Now permanent infusion was introduced with a *very small quantity of adrenaline* and the blood pressure of animal A was thus kept within physiological boundaries. Through this experimental arrangement it was possible to decide whether deep narcosis of

the brain, without attaining the periphery, lessened the basal metabolism increased by thyroxin or not. The result is shown by Tables XXIII and XXIV.

In not a single case was there a drop in the O_2-consumption after elimination of the brain function, so that we have to conclude that the increase of metabolism produced by the thyroid is conditioned purely peripherally *and is not even partly a result of increased innervation. Operative disconnection of the brain* followed by vagotomy on cats led to the same result. (See Table XXV.) We proceeded like Issekutz, with one important difference, that we used much smaller dosages of adrenalin than he did.

Thus, it was demonstrated that neither elimination of the brain, nor intersection of the vagi, led in any single case to a decrease of basal metabolism increased by thyroxin.

TABLE XXIV
Changes of O_2-consumption.

Experiment	By Thyroxin %	By deep narcosis of brain centres %	Time after luminal injection hr.	min.
1	+44·4	— 2·2	2	25
2	—	— 2·2	—	
3	+35·8	— 6	2	20
4	+42·7	— 6	2	40
5	+34·4	± 0	1	25

Looking for the cause of this contradiction among the results of the experiments, we established that it was conditioned by the fact that Issekutz used such high dosages of adrenalin for maintaining the blood pressure that they were bound to lead, as Hari[113] was the first to find out, to a powerful reduction of the O_2-consumption in curarized animals. If the blood pressure was, however, kept high—as in our experiments—with very small quantities of adrenalin, then neither the deepest possible narcosis of the brain centres nor their operative elimination can bring about a decrease of combustion. We wish to demonstrate this metabolic effect of adrenalin, which looked like a central thyroxin effect in Issekutz's investigations, in a few experiments.

As mentioned, our experiments differ from those of Issekutz only in that we kept the blood pressure at a normal level with much smaller quantities of adrenalin. For this purpose, we arranged for the infusion from two connected small dropping-glasses (Mariotte), of which one was filled with Ringer's solution and the other with a 10^{-5} adrenalin solution. Through a suitable mixture of both solutions it is possible to maintain the blood pressure at a normal level for hours with less than 1 γ adrenalin per kg./min., which, of course, is of no account for the O_2-consumption.

TABLE XXV

Effect of decerebration and vagotomy on the O_2-consumption of cats, increased by thyroxin.

Experiment No.	W Date	Before decerebration				After decerebration									Difference %
		V normal	V after Thyroxin and Curare	θ P	Increase %	T γ	θ P	V	T γ	θ P	V	T γ	θ P	V	
1	1·6 19 X – 29 X 37	1532	2028	39·0°C 100	32	30′ 0·5	39·7°C 92	2124	1h 40′ 0·5	39·1°C 82	1932	2h 40′ 0·5	39·2°C 80	1932	—4
2	1·9 23 X – 4 XI 37		1992	39·2°C 80		1h 10′ 0·8	39·5°C 78	1908	1h 30′ 0·8	39·3°C 70	1824	1h 50′ 0·8	39·4°C 70	1764	—11
3	3·2 1 XI – 8 XI 37	2376	2820	39·4°C 94	18·7	60′ 1·2	39·3°C 70	2676	2h 00′ 0·9	39·2°C 78	2580	2h 30′ 0·9	39·4°C 90	2652	—6
4	2·1 23 X – 5 XI 37	1354	1776	39·5°C 72	31	50′ 0·9	39·3°C 64	1740	1h 10′ 0·9	39·2°C 73	1716				—3
5	2·2 3 XI – 13 XI 37	1244	1476	38·4°C 100	18·6	60′ 2·0	38·4°C 96	1464	2h 25′ 2·0	38·4°C 96	1404	2h 50′ 2·0	38·5°C 90	1404	—5
6	2·4 28 XI – 4 XII 37		1680	39·0°C 90		60′ 0·8	39·1°C 90	1560*	2h 10′ 0·8	39·1°C 90	1560**	3h 00′ 0·8	39·1°C 88	1644	—2
7	2·5 28 XI – 5 XII 37		1860	39·1°C 150		1h 50′ 1	39·4°C 150	1848*	3h 00′ 2·1	39·2°C 140	1824**	4h 20′ 2·1 2·7	39·0°C 148	1824	−1·9

V = O_2-consumption in cc. per hour.
P = arterial blood pressure in mm. Hg.
γ = infused quantity of adrenation in γ per kg. per minute.
T = time after decerebration in hours and minutes.
θ = body temperature in °C.
W = weight of animal in kg.
* Vagi intact.
** Vagotomized 1 hr. 10 min. previously.

In Diagram 20, we demonstrate an experiment made on a dog, which received preliminary thyroxin treatment, with crossed brain circulation.

The O_2-consumption of the animal remained constant for two full hours, during which period the adrenalin administration remained under 5γ. At the beginning of the third hour the adrenalin quantities were increased to 20 γ, and the O_2-consumption sank from 375 c.c. to 250 c.c., i.e., 36 per cent.

We obtained the same result on normal animals whose brain was eliminated, and show one such experiment in Diagram 21.

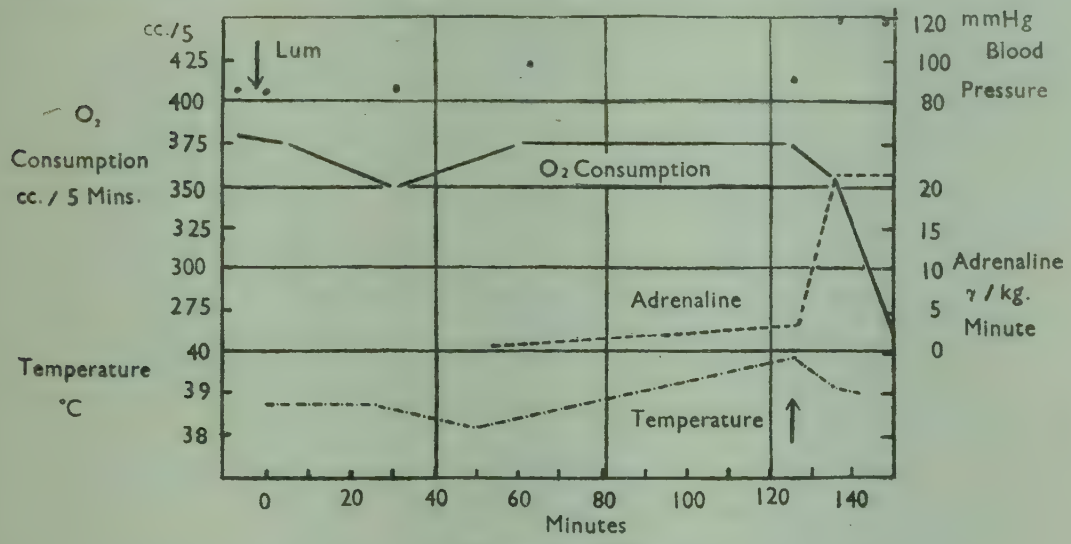


DIAGRAM 20
Thyroxin-pretreated dog with crossed brain-circulation.
At ↑ 1 mg. adrenalin.

Here as well, concurring with Hari's experiments and those of Antal and Schleinitzer,[114] the interconnection between adrenalin quantities and O_2-consumption is undeniable.

These experiments are decisive not only for the purely peripheral metabolic effect of thyroxin. They also create doubts in respect of the very existence of the so-called metabolic centres, from which *nervous impulses* are supposed to pass into the organ cells, in order to keep the combustion high therein. Our experiments with crossed brain circulation, in which the intact brain of the animal was connected with the curarized body through vegetative fibres in the vagus and sympathetic chain, are particularly impressive in this respect. Before administration of the narcosis, the head of the animal often showed the greatest irritation without any influence on the O_2-consumption of the body—in which the musculature was paralysed against central impulses. Neither did a decrease of the O_2-consumption take place, if these centres were narcotized or separated. It is, therefore, impossible to speak either of a vegetative " chemical organ tonus " or of a release or increase of such impulses through thyroxin.

It seems, however, that our results were not yet sufficient to abandon the centralistic theory of the thyroxin effect on basal metabolism, for recently Issekutz, jun., and Komlos tried again to provide the evidence that narcosis of the metabolic centres diminishes, if only temporarily, combustion increased through thyroxin. A report was made at the annual conference of the Hungarian Physiological Society at Kolozsvar in September 1942. They appear to have tried to reduce the increased O_2-consumption caused by thyroxin by a combination of 0.03–0.02 g. morphine and 0.005 g. scopolamine per kg. rabbit. In order to eliminate the

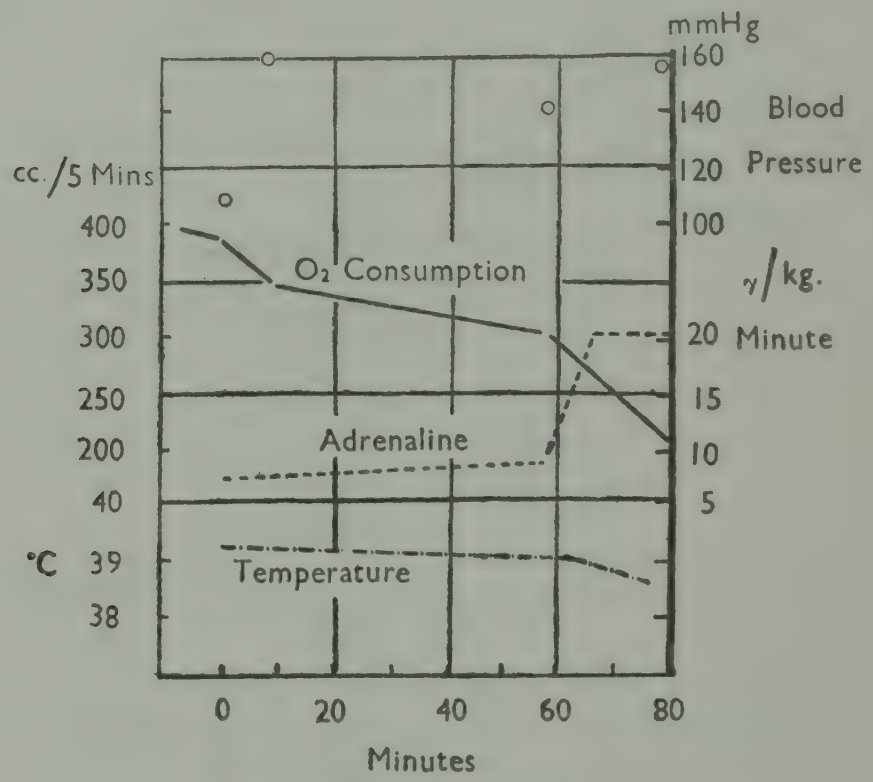


DIAGRAM 21
Curarized normal dog. Medulla oblongata dissected.

spontaneous movements of the animals, they administered quantities of urethane which had no effect on metabolism. The authors saw a temporary diminution of the O_2-consumption—increased through thyroxin— for about an hour, which was not conditioned either by a drop in blood pressure or by a lessening in breathing volume, and regarded it as a renewed "proof" that thyroxin increases combustion through irritation of the metabolic centres.

It is impossible to make this conclusion coincide with the results of our experiments, according to which deepest narcosis of the central nervous system and complete elimination of the brain do not affect the thyroxin-increased O_2-consumption, because the morphine-scopolamine narcosis of the metabolic centres cannot possibly be more effective than excision with the knife. How-

ever, it is known that scopolamine paralyses not only the centres but also the periphery, and it was therefore natural to suppose that it might be a peripheric influence which diminishes the increased metabolism. It was not difficult to demonstrate that Issekutz's findings had nothing in common with a central paralysis, when we compared, after similar dosages of morphine-scopolamine, *the gas exchange increased through dinitrophenol,* and the normal gas exchange of rabbits. My experiments in association with

DIAGRAM 22.

Effect of morphine-scopolamine on the gas-exchange of rabbits.

—— O_2-consumption.

- - - - CO_2-production.

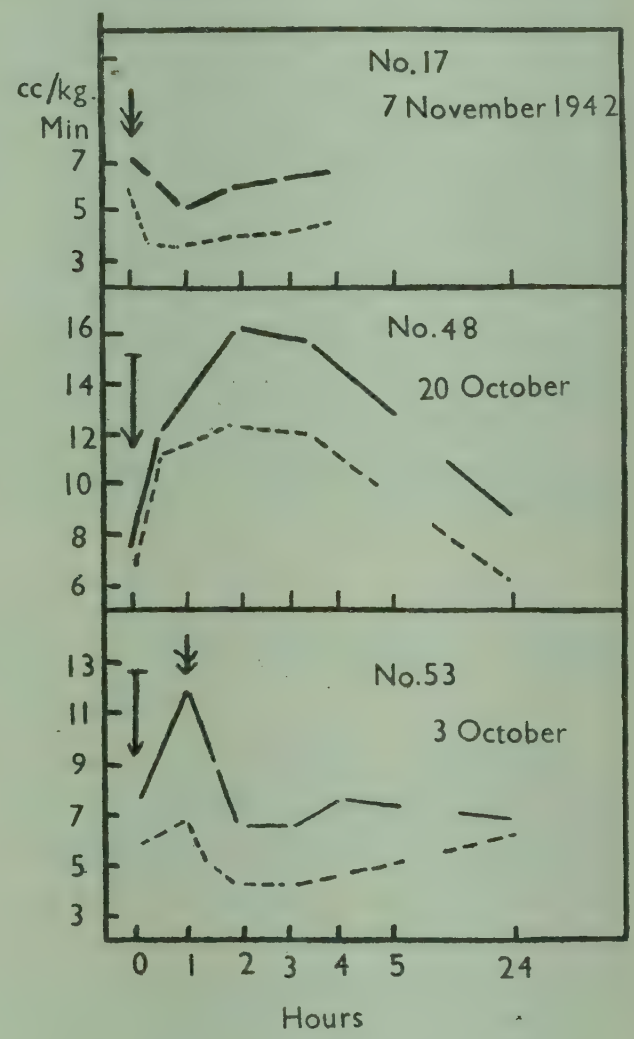
↓ 0.02 gm./kg. α-dinitrophenol subcutaneously.

↓↓ 0.03 gm./kg. morphine and 0.005 gm./kg. scopolamine intravenously.

No. 17. Effect of morphine-scopolamine.

No. 48. Effect of α-dinitrophenol.

No. 53. Effect of morphine-scopolamine after α-dinitrophenol.



Eszter Meszaros demonstrated that both the increased O_2-consumption after dinitrophenol, which attacks peripherally, and the uninfluenced O_2-consumption of normal rabbits are powerfully decreased through morphine-scopolamine, which corresponds to what Issekutz and Komlos observed after thyroxin. (See Diagram 22.)

Once again the stubborn supporters of the centralistic theory of the effect of thyroxin were misled in that they did not pay attention to the peripheral influence of narcotics. In the older experiments it was the over-large dosages of urethane and luminal which, in addition to the central-narcotic effect, also paralysed

cell oxidation. This time it was probably the addition of scopolamine with its peripheral point of attack similar to that of atropine. These newest experiments of Issekutz did not provide any support for the theory that thyroxin increases combustion through stimulation of metabolic centres.

Facts are not available regarding other substances which may increase combustion in the vegetative organs through irritation of the metabolic centres as a result of nervous impulses. Only for the muscle could it be established (Mansfeld and Lukacs[115]) that it possesses a " chemical tonus " transmitted through sympathic nerves, which is abolished by narcosis, as can be concluded from Nakamura's[116] experiments. The very existence of *this* direct central-nervous effect on combustion processes was violently disputed by Dusser de Barenne,[117] but later recognized[118] on the grounds of his own experiments.

These experiments of ours, with crossed brain circulation, showed no such effect of the *brain* on combustion, so that its decrease after denervation of the hind legs—regularly found in our former experiments—points to the possibility that this " chemical " muscle tonus, similarly to the " mechanical " one, is *reflectorily conditioned and conveyed by the spinal cord.*

(*b*) The Novocaine-Thyroxin Antagonism and a Newly Recognized Role of Thyroxin in Relation to Physical Heat Regulation

Glaubach and Pick[103] have established the following interesting fact. By injecting a guinea-pig with novocaine (0.20 gm./kg.) a considerable fall of temperature lasting a few hours is obtained; but when thyroxin is injected for three days running prior to the novocaine experiment, then novocaine does not bring about any fall of temperature worth mentioning. Thyroxin, however, is capable of preventing the drop in temperature only when the connection of the body with the brain is intact. Should the spinal cord at D 5–D 6 be intersected, then thyroxin cannot prevent the drop of temperature through novocaine.

The authors supposed tacitly that novocaine brings about a decrease of the body temperature in *that it primarily paralyses combustion* and therefore they concluded, erroneously, as we shall see, that thyroxin prevented the fall of temperature because of its known effect on metabolism. As this effect takes place only in animals which have their spinal cord intact, they drew the conclusion that, besides direct peripheral cell effects, *the central nervous system plays an important role* in the chemical processes instigated through thyroxin.

This conclusion, which contradicted our experiments as well as those of Oberdisse and Roda,[97] according to whom thyroxin displays its oxidative effect undiminished after intersection of the spinal cord, had to be thoroughly analyzed. This I did in conjunction with Nils Alwall and Irene Scheff-Pfeifer.

In the first place we investigated more closely the effect of novocaine on temperature in order to find out whether it is brought about, as Glaubach and Pick accepted, by primarily diminishing combustion. For this purpose we examined the effect of novocaine on the basal metabolism of guinea-pigs at a temperature at which novocaine does not lead to a fall of temperature, namely at 33° C. This was obviously necessary in order to find out whether novocaine diminished the temperature by preventing oxidation or by increasing loss of heat. In the second place, *if we worked at a low external temperature and the animal cooled off as a result of an increased loss of heat*, the combustion at the low body temperature would have correspondingly diminished, which might have been responsible for an erroneous assumption of a metabolic effect of novocaine, as in the case of Issekutz and Kövári.[120]

The result of our metabolic experiments is demonstrated in Table XXVI.

TABLE XXVI

Animal No.	Weight gm.	Preliminary Periods: CO_2 production in mg. per gm. animal per hr.			Novocaine Experiment: Body Temperature °C		Novocaine Experiment: CO_2 production in mg. per gm. animal per hr.	Difference %
		1	2	3	before Novocaine	after Novocaine		
1	360	1·33	1·38	1·25	36·7	36·7	1·20	—4
2	375	1·44	1·25	1·17	37·7	37·6	1·19	0
3	500	1·15	1·01	1·04	38·0	37·0	1·02	0

The experiments demonstrated that novocaine did not diminish combustion when a fall of temperature through elevated external temperature is prevented. The fall of temperature is therefore not conditioned through a paralysis of heat production.

This led to the suspicion that novocaine leads to a fall of temperature through a *paralysis of physical heat regulation*, i.e., an increased loss of heat, and that the antagonistic effect of thyroxin, which is eliminated by intersection of the spinal cord and is thus centrally conditioned, is directed not on *production of heat* but on *loss of heat*. This suspicion was further strengthened in that the antagonistic effect of thyroxin *can be destroyed through dosages of ergotamine which have no effect on metabolism, i.e., elimination of the vasoconstrictors as well as through section of the spinal cord.*

In Table XXVII we show that the dosages of ergotamine which we used did not affect the metabolism of guinea-pigs which was increased through thyroxin; in Diagram 23 we demonstrate that they destroy the novocaine-antagonistic effect of thyroxin in the same way as section of the spinal cord.

TABLE XXVII

Animal No.	Weight gm.	Metabolism : mg. CO_2 per gm. of animal per hour			Difference after		Dose of Thyroxin : mg. per 100 g. of animal
		Normal	after Thryoxin	after Ergotamine	Thyroxin %	Ergotamine %	
1	347	1·42	1·92	1·82	+35	— 5	0·25
2	309	1·44	1·87	1·60	+30	—14	0·29
3	340	1·19	1·82	1·84	+53	0	0·67
4	318	1·32	1·83	2·00	+38	+ 9	0·72

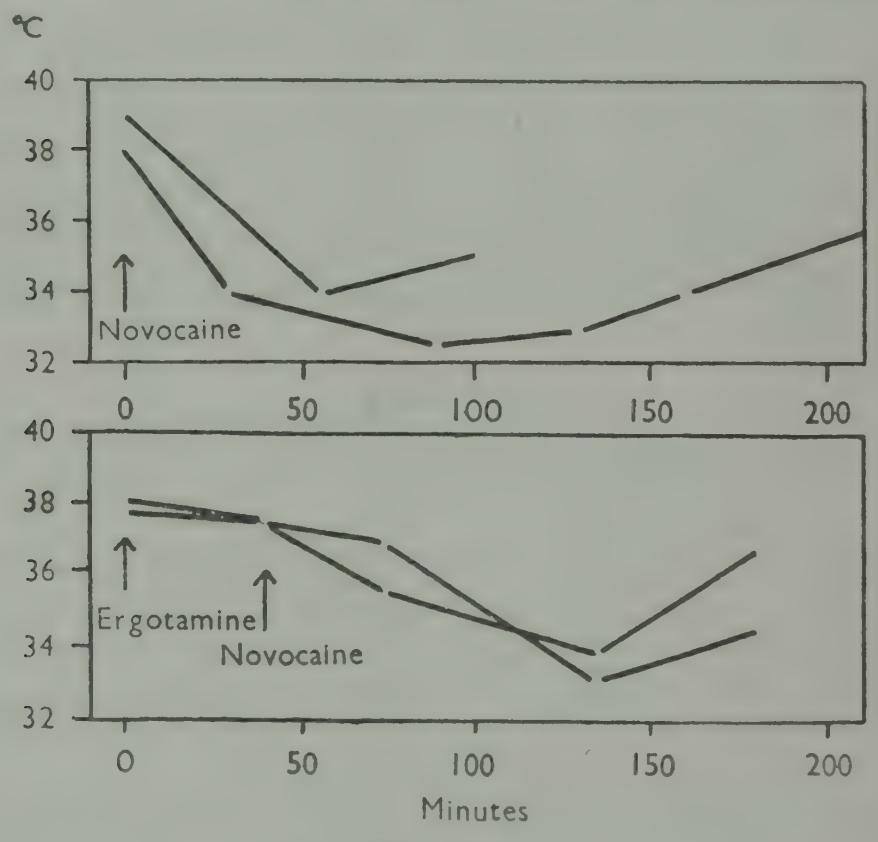


DIAGRAM 23

Above: Two guinea pigs with their spinal cords dissected.

Below: Two guinea-pigs with their spinal cords intact.
All the four animals received for the last three days before the novocaine experiment 0.3 mg. thyroxin daily. Ergotamine inhibits the thyroxin effect as does the section of the spinal cord.
Ordinate: Body temperature.

These facts could only be interpreted in the following way. Thyroxin does not prevent a fall of temperature caused by novocaine through its metabolic effect, but through a hitherto unknown central effect on the vasoconstrictors, which come out of the spinal cord below D 5, and are interrupted, therefore, through a section

at this level, and paralysed through ergotamine like vasoconstrictors in general.

If this interpretation is correct, it would mean the discovery of another effect of thyroxin. This hormone *would then have an additional function to its peripheral effect on heat production by interfering with the physical heat regulation through a central-nervous effect on the loss of heat.*

Bearing in mind the importance of this conclusion, this effect of thyroxin was subjected to a further analysis. Together with Eszter Meszaros[121] I tested thermo-electrically both the effect of novocaine on the loss of heat and the antagonistic effect of thyroxin.

The thermo-elements we used for measuring simultaneously the temperature of rectum and skin on guinea-pigs were made of iron-constantan. The insulated electrodes were suspended in boiling ether, the rectum electrode was shut off in a very thin-walled glass tube. The skin temperature was taken on the carefully shaved back above the rump-bone—a part of the body which is very poor in muscles. The electrodes—covered with a thin insulating layer of shellac—lay in a groove of a 2 cm.-thick paraffin block, corresponding to their thickness, which covered fully the skin surface to be measured and insulated it thermally from the environment. The animals were fastened lightly on a board in a squatting position. As an instrument for measuring we used a highly sensitive mirror-galvanometer with a scale distance of 2 metres. 0.01° C. corresponded to a deflection of 2 mm.

The skin temperature at a constant exterior temperature gives a good measurement of the content of blood in the skin. When this amount augments through vaso-dilatation, the skin temperature augments as well, and the difference between the internal and external temperature decreases. When the loss of heat is so powerful *that the production of heat cannot keep pace with it, as in the case of the effect of novocaine,* then the temperature of the blood naturally drops, and with it the skin temperature. The increased content of blood manifests itself in a constant decrease of the difference between the temperatures of the skin and the rectum.

That novocaine increases the loss of heat, as was to be expected following the metabolic experiments, is shown in Diagram 24. On its right half can be seen distinctly the original increase of the skin temperature and then the decrease of the difference between the internal and external temperatures in three novocaine experiments.

If the fall of temperature due to novocaine was brought about through paralysis of heat production, then, on the contrary, a drop of the skin temperature parallel to the body temperature should be observed.

In the experiments we made on animals which had preliminary thyroxin treatment, we saw distinctly that novocaine *did not effect any increase of the skin temperature,* that, correspondingly, no drop occurs in the temperature of the body, and that, later, no decrease has been seen in the difference of the temperature between skin and rectum. Had thyroxin prevented the fall of temperature through increased heat production, as Glaubach and Pick assumed, then an increased loss of heat would manifest itself unchanged after novocaine in a thyroxin-treated animal also.

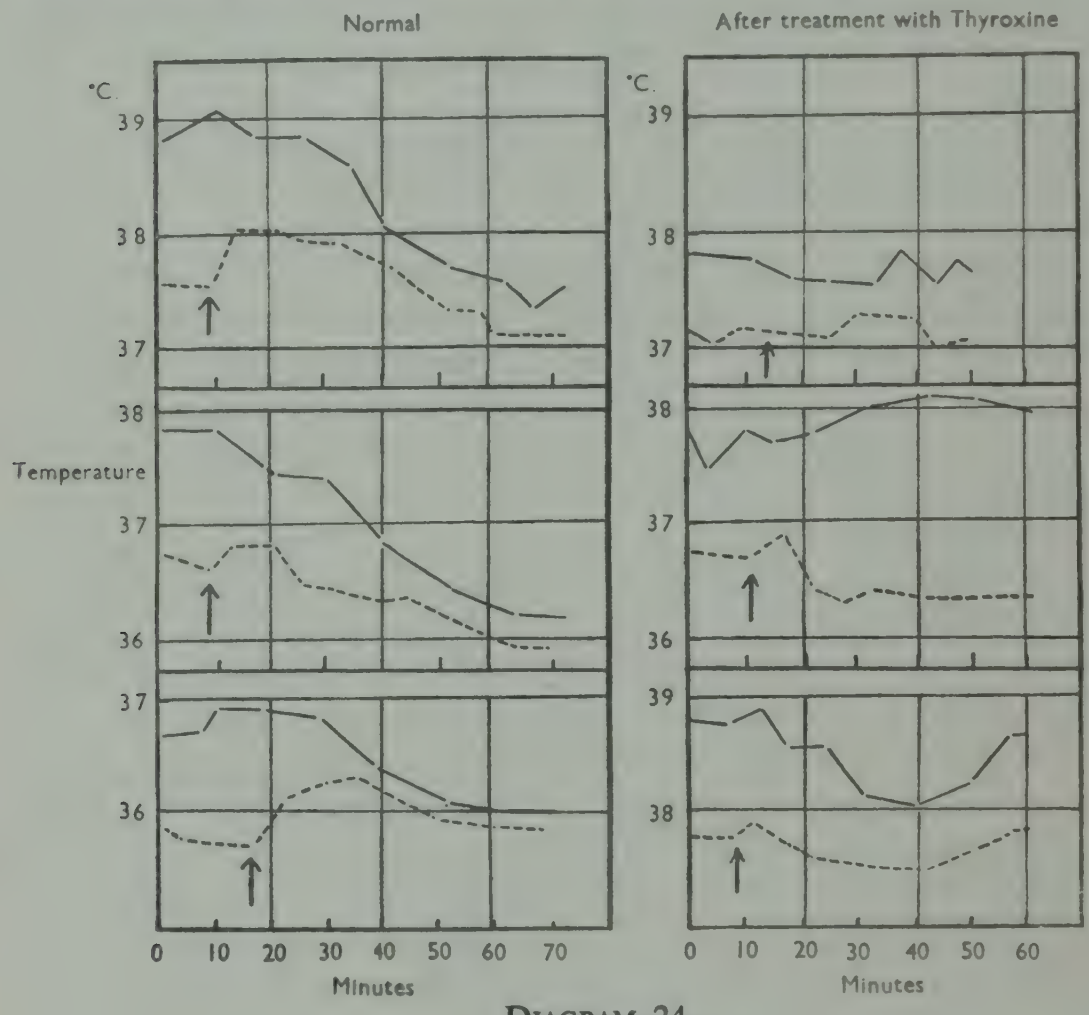


DIAGRAM 24.
At ↑ 0.01 gm. novocaine per 100 gm. body weight.
—— Rectal temperature. - - - Skin temperature.

That this assumption is correct can be proved in the following way: We imitate an increased heat production by sewing a small glow-lamp in the abdominal cavity of the animals and keep the temperature of the animals constant during the novocaine-experiment by switching the current on and off.

In Diagram 25 we see the contrary effect of thyroxin and of heat supply on the temperature of the skin in the novocaine experiment.

In all four experiments the temperature of the body remained unchanged. After administration of thyroxin the constancy of

temperature depends on the prevention of the novocaine-conditioned widening of the vessels in the skin; whereas in the experiments in which constancy is achieved through heat supply, we saw, on the contrary, a considerable increase of the skin temperature after novocaine.

These experiments showed distinctly that thyroxin prevented the fall of the temperature by novocaine not through increased heat production but through throttling the loss of heat. What does not seem clear at first is why thyroxin *did not increase*

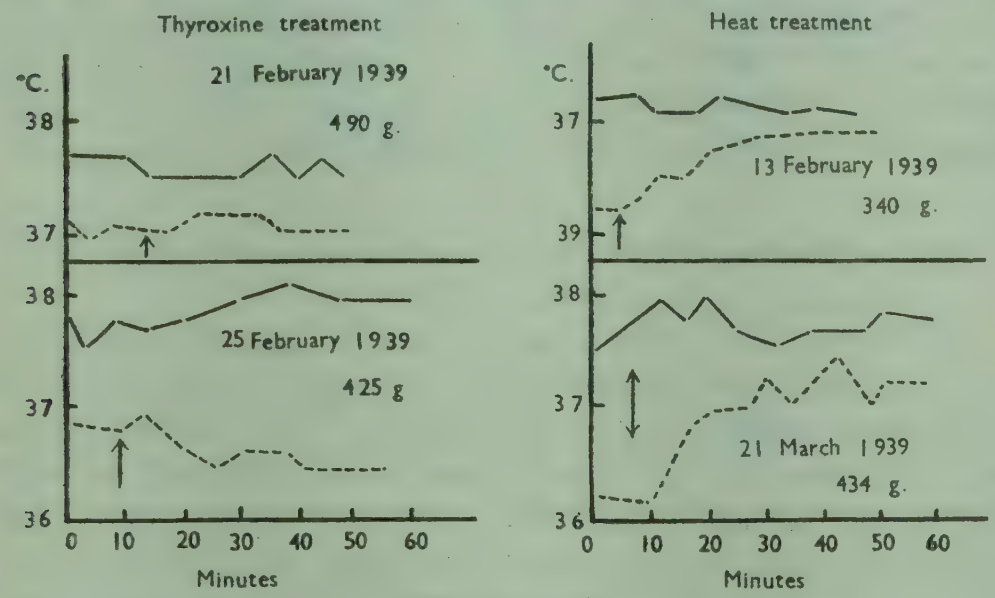


DIAGRAM 25.

At ↑ 0.01 gm. novocaine per 100 gm. body weight.
—— Rectal temperature. - - - Skin temperature.

the heat production in these experiments as well, though this metabolic effect of thyroxin has been long known and undisputed. We shall deal with this question fully in Chapter XI. Here we shall only mention that both the experiments of Glaubach and Pick, in which they saw the novocaine-antagonistic effect of thyroxin disappear after section of the spinal cord, and our own confirmative investigations happened to be made during the warm season, in which, as we shall show later, the quantities of thyroxin used did not suffice to bring about an *increase in heat production.* The central effect on the physical heat-regulation, which we have just described, becomes apparent and disappears only after intersection of the spinal cord or ergotamine.

If, however, similar dosages of thyroxin are administered in winter, or a triple quantity in summer, then the *heat production* is increased through thyroxin *to such an extent* that, despite an augmented loss of heat through novocaine, the fall of temperature is only slight. Also, as we shall see further, this thyroxin effect, being peripherally conditioned, cannot be influenced through intersection of the spinal cord or ergotamine.

The assumption of Glaubach and Pick was right, inasmuch as it is a matter of a central thyroxin effect. This effect is, however, basically different from the one which increases combustion.

An analysis of the novocaine-thyroxin antagonism led to the conclusion that the increase of metabolism through thyroxin is conditioned purely peripherally; the assumption that thyroxin increases combustion through stimulation of the metabolic centres has, at present, no experimental foundation whatsoever.

On the other hand, this analysis enriched us with the knowledge that thyroxin, while hindering the loss of heat through its effect on the vasomotor centres, i.e., influencing physical heat-regulation, interferes actively with the heat-balance. Its role in chemical heat regulation will be discussed later.

(*c*) The Alleged Effect of Thyroxin on the Centres of the Mid-Brain

It is known, as has been mentioned, that thyroxin displays its effect on combustion with a latent period of 24 hours. It was, therefore, most surprising to learn from L. Stern's collaborators[105] that if, by avoiding the blood-brain barrier, thyroxin or watery extracts, the so-called metabolites of cats' thyroid, are injected directly into the lateral ventricle of dogs, the O_2-consumption, which is constantly measured, is powerfully increased as soon as 2–3 hours after. The authors regarded this as proof that thyroxin stimulates directly the metabolic centres and thereby increases combustion. Upon examination of their experiments we found the following:

The method itself, namely, an injection of chemical substances into the lateral ventricle, is described by L. Stern and colleagues[122] as highly fruitful; we should like to point out that many years ago Jacobi and Roemer[123] had already demonstrated that any foreign substance brought into the lateral ventricle, even metallic mercury, which causes only slight reaction, acts in the same way as a puncture of the thalamus and leads within a few hours to an increase of several degrees in temperature, which goes hand in hand with vaso-constriction in the skin and tremor of the muscles. By rigorous observation of the prescriptions of the Stern method, when we brought into the lateral ventricle 0.5 mg. thyroxin-Roche and measured the body temperature uninterruptedly, we saw for ourselves that this is the case also after injections of thyroxin into the lateral ventricle. In Diagram 26 we see the course of temperature in three such experiments.

It is known to anybody who ever made correctly the puncture of the thalamus on a dog, that here tremor of the muscles is very

discernible, and we, therefore, cannot understand how it was possible that this tremor should have escaped the attention of L. Stern and his pupils.

Tremor of the muscles makes it impossible to determine the basal metabolism of an animal at this state of increasing temperature, but one could say nevertheless that it presents a central effect of thyroxin directed on the heat-centre in the sense of Issekutz's hypothesis and leads via this heat-centre to an increase of combustion. The question arose whether we had to deal here with a specific effect of thyroxin which would be of interest and importance for both normal and pathological heat regulation. To examine this, we investigated first whether the solvent of Hoffmann-La Roche's thyroxin produces fever also when brought into the lateral ventricle. This solution is, as is known, a N/100 NaOH solution. We injected 0.5 c.c. of this solution into the lateral ventricle of dogs. Diagram 27 demonstrates the effect of such an experiment.

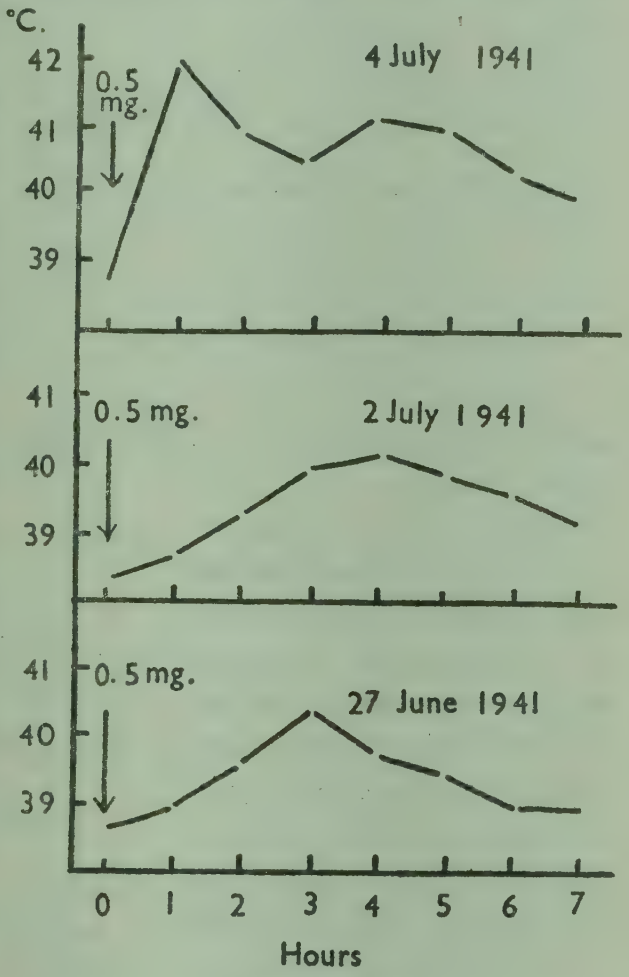


DIAGRAM 26.
Fever after injection of 0.5 mg. Thyroxin-Roche in lateral ventricle of dogs.

It was demonstrated that the fever produced by the thyroxin injections did not represent a specific effect of thyroxin at all, but had already been brought about by the use of the NaOH solution. It was also shown that the application of various concentrations of NaOH presents an excellent method of bringing about distinctly graduated fever reactions; this is not possible with puncture of the thalamus. Injections of 0.5 c.c. N/10 NaOH solution led to an increase of fever up to 42° C., and the fever lasted for two days.

The authors may rightly say that they used not only thyroxin dissolved in alkali, but also "thyroid-metabolites" of cats, i.e., watery extracts of the thyroid glands of cats prepared at body temperature. These naturally contain various proteins, partly of the thyroid gland, partly of the blood serum, and the authors

claimed that these metabolites were particularly effective when the utilized thyroids were rich in blood serum. We wished to see which was responsible, whether thyroxin or other thyroid substances, or perhaps, proteins of the used organ, or serum somewhat alien to the lateral ventricle and the centres situated within its walls. So, for injections into the lateral ventricle of dogs, we used blood serum of the same kind and to meet the objection that the serum might contain thyroid substances as well: we utilized blood serum of a dog which had been thyroidectomized several weeks earlier. *This blood serum of a thyroidectomized dog injected into the lateral ventricle of normal dogs caused high fever with tremor of the muscles.* (See Diagram 28.)

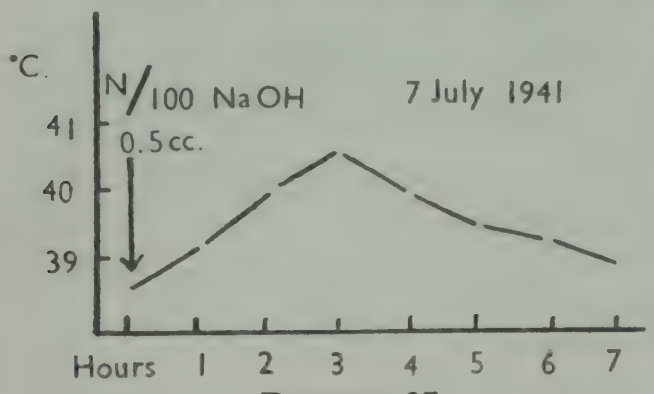


DIAGRAM 27.

Fever after injection of 0.5 c.c. N/100 NaOH in lateral ventricle of dog.

These experiments confirm the findings of Jacobi and Roemer,[123] according to which every irritation of the walls of the lateral ventricle provokes fever, and show that the method recommended by L. Stern, of injecting substances into the lateral ventricle for the examination of metabolic processes, is misleading and therefore unsuitable.

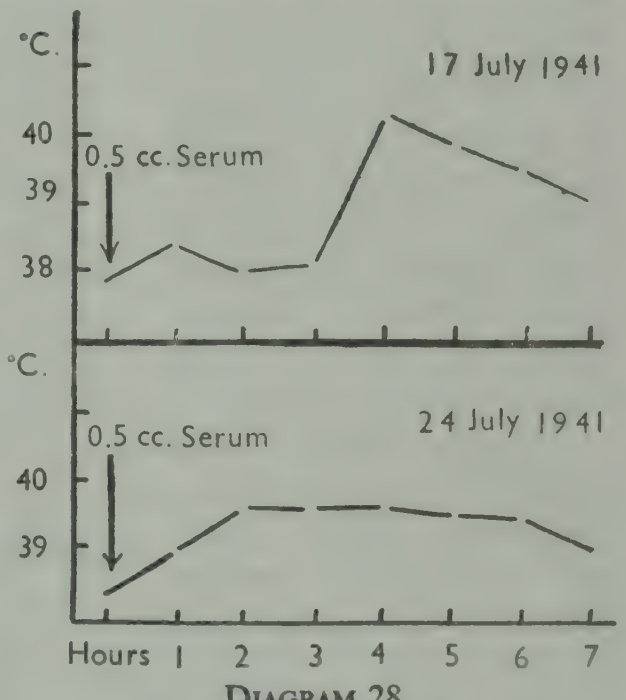


DIAGRAM 28.

Fever after injection of blood serum of thyroidectomized dog in lateral ventricle of normal dog.

It was demonstrated that the experiments made by L. Stern's pupils in order to decide the question whether thyroxin increases basal metabolism centrally or through a peripheral point of attack were wrong; because the fever produced through the injection and the tremor of the muscles which follows thereafter makes an examination of basal metabolism impossible. The question remained open whether, with a rightly chosen method, a direct effect of thyroxin on the centres could be proved.

To examine this, we repeated the experiments of the Russian authors *on curarized dogs*, as curare is known to prevent tremor of the muscles but at the same time does not disturb in the slightest the metabolic effect of thyroxin, as we have seen. (Cf. Table XXIII.) The results of our experiments on curarized animals are shown in Table XXVIII.

TABLE XXVIII

Effect of Injection of 0.5 mg. Thyroxin-Roche into Ventricle of Curarized Dog on Gas Exchange.

No.	Date	Time before or after injection of thyroxin	cc. O_2 per kg. per min.	cc. CO_2 per kg. per min.	% difference		Blood pressure mm. Hg
					O_2	CO_2	
1	24 VI 1941	before	9·9	10	—	—	184
		½ hour after	7·6	6·6	—23	—34	180
		1½ hours after	6·8	6·7	—22	—33	180
2	26 VI 1941	before	8·1	7·4	—	—	140
		1½ hours after	6·6	6·2	—19	—17	160
		2½ hours after	9·2	8·2	+13	+10	156
		3½ hours after	8·9	7·7	+ 9	+ 4	164
3	30 VI 1941	before	7·6	6·9	—	—	170
		1 hour after	7·5	6·6	— 2	— 5	158
		2 hours after	7·7	6·7	+ 1	— 3	162
		3 hours after	7·9	6·3	+ 4	— 9	162
4	3 VII 1941	before	7·5	6·1	—	—	142
		1 hour after	7·2	5·2	— 4	—15	144
		2 hours after	8·1	6·2	+ 7	+ 1	132
		3 hours after	8·0	5·7	+ 6	— 7	142

We see that, when tremor of the muscles is prevented through curare, thyroxin injections into the lateral ventricle do not increase combustion even once within three and a half hours.

We come to the conclusion that experiments with a direct effect of thyroxin on the brain centres do not offer support for the view that this hormone increases basal metabolism by stimulation of metabolic centres.

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CHAPTER IX

THE PASSAGE OF THYROXIN THROUGH THE NERVES

ALTHOUGH the large number of reported experiments leaves no doubt that thyroxin increases combustion through a direct effect on the cells, we came, during our investigations, across a fact which makes a participation of the nervous system likely in the production of the thyroxin effect.

We mentioned already that if we remove animal organs or organ fragments before and 24 hours after the thyroxin injection and test them as to their O_2-consumption according to Warburg's method, an increase of the O_2-consumption is noticeable in the later-removed organ as a result of thyroxin. This experiment in itself is a strong proof for the peripheral point of attack, because the increased combustion in the surviving organ continues after its separation from the nervous system; but we wanted to find out what happens if we use organs which had been separated from the nervous system, i.e., denervated prior to the thyroxin injection.

For this purpose we denervated completely *both* kidneys in a rabbit and painted the nerve stumps on the kidney with 5 per cent phenol. After a waiting period of one to two weeks, we removed one of the denervated kidneys, prepared sections from it, after Warburg, and tested the O_2-consumption. After the nephrectomy 1 mg. thyroxin was injected, and 24 hours later the second kidney was treated in the same way.

As pointed out above, the best way to reach the thyroxin effect in Warburg's experiment is by introducing an anaerobic period prior to the actual experiment or by giving ethylcarbylamine which paralyses Pasteur's reaction. In a series of experiments the slices had a preliminary treatment in this way, which has the disadvantage that the vitality of the cells is damaged and therefore furnishes smaller O_2-values than under normal circumstances. Thereafter we made a second series of experiments in which the kidney slices were tested without such preliminary treatment.

Only on one occasion did we see on the denervated kidney an increase of the O_2-consumption which went beyond the limit of error, whereas on the non-denervated kidney the thyroxin increase occurred in all experiments but one. (No. 63, Table XXIX, p. 77.)

Whilst, on the one hand, the continuation of the thyroxin effect after isolation of the organ proved the peripheral point of attack,

we found, on the other, after intersection of the nerves the thyroxin effect in the organ cells is absent, i.e., the nerve supply is a preliminary condition for the development of the peripheral effect of thyroxin, which continues after isolation of the organ.* To clarify this contradiction we used a hypothesis and assumed that possibly thyroxin does not penetrate the organ cells directly from the blood, but is first absorbed by the central nervous system, and penetrates the organ cells after travelling through the peripheral nerves—like the tetanus toxin. (Meyer and Ransom.[124])

In harmony with this hypothesis was our earlier finding that thyroxin does not accelerate combustion directly, but accelerates enzyme action, and penetrating from the blood cannot exercise its effect on the cell surface, which is the seat of combustion, because the substrate of its effect, enzyme action, is suppressed by combustion. We thought that if thyroxin is to become effective in the cell, it must avoid the cell surface and get into that oxygen-poor interior, where the nerve unites with the cell and the enzyme action takes place; we know from the physiology of the muscles that it is not combustion but the enzyme action which is set in motion through nerve stimulation. Another support for our assumption was the fact that thyroxin does not immediately display its oxidation-increasing effect in the organism but only after a latent period of 20–24 hours, which can probably be explained by the fact that thyroxin has to cover long distances in the nerves before reaching the cell. The finding of Schittenhelm and Eisler,[125] according to which the iodine content of the mid-brain and the tuber cinereum increases after administration of thyroxin, also points to an affinity of the nerve substance to thyroxin.

Our assumption was strengthened by the fact that the living nerve is doubtlessly capable of transmitting chemical substances. This was demonstrated for the tetanus and diphtheria toxins by the investigations of Meyer and Ransom,[124] for the virus of herpes and rabies by those of E. W. Goodpasture,[126] for the virus of infantile paralysis by R. W. Fairbrother and E. W. Hurst,[127] as well as by the investigations of A. Doerr[128] and his colleagues (S. Seidenberg and Fl. Magrassi). That physiological substances can reach the seat of their effect through the nerve results from the interesting fact, which is well known to hunters, that after injury to or loss of a testicle in a stag, its antlers are stunted uni-

* It is true that in the experiments made by Oberdisse and Roda, the authors could not detect any influence of the nervous system on the development of the thyroxin effect, but their experiments differed from ours in that they denervated only one kidney (an essential difference), namely, the one exposed to the effect of thyroxin; thus, they always compared an intact kidney with a denervated one, and this may have obscured their results.

laterally, and this always counter-laterally, which no doubt points to the passage of the active substance of the testicle through those paths of the spinal cord which are traversed. Laszlo Nagy writes in the *Hungarian Journal for Hunters* for the year 1931–33: "The development of the antlers is linked up with the genital organs. If the stag is castrated on one side or if a testicle is injured (through a shot, for instance) the antlers do not grow on the opposite side." Adolf Balkay writes in his work, *The Stag and its Hunting* (1903), p. 31 (in Hungarian): "If only one of the testicles perishes, then only one branch of the antlers, the one of the

TABLE XXIX

Qo_2 Values of Normal and Denervated Kidneys of Rabbit before and after Injection of Thyroxin.

Experiment No.	Thyroxin injection mg.	Qo_2 1st kidney	Qo_2 2nd kidney	Difference %	With or Without Preliminary Treatment (see text)
57	—	4·6	4·3	— 3	1 With
58	—	4·3	4·5	+ 4	
59	1 × 1·0	3·0	4·1	+37	
60	4 × 0·5	3·9	4·6	+18	
61	4 × 0·5	5·6	6·7	+20	
62	3 × 0·5	8·31	12·49	+50	2 Without
63	3 × 0·5	9·26	9·25	± 0	
64	4 × 0·5	9·59	11·21	+17	
65	4 × 0·5	10·1	14·3	+42	
		DENERVATED			
66	—	4·5	4·9	+ 9	1 With
67	—	3·5	3·8	+ 9	
68	—	5·4	5·6	+ 3	
69	4 × 0·5	5·8	5·7	— 2	
70	4 × 0·5	6·2	5·9	— 4	
71	4 × 0·5	5·9	6·1	+ 4	
72	3 × 0·5	8·36	8·62	+ 3	2 Without
73	4 × 0·5	10·20	12·11	+20	
74	4 × 0·5	10·18	11·34	+11	
75	3 × 0·5	10·61	10·54	± 0	

opposite side, is stunted." In Dombrowsky's book, *The Formation of the Antlers,* we read: "The stunting and malformation of the antlers as a result of injuries to the testicle never appear on the side of the injury but always on the other side, transversely."

All this seemed to justify a thorough analysis of our assumption and so we investigated:

(*a*) Whether the nerves of cold- and warm-blooded animals possessed in fact the faculty of conducting thyroxin and bringing it into the interior of the cells.

(*b*) Whether thyroxin reaches the organ cells through the nerves under physiological conditions.

(*a*) The Faculty of the Nerves to Convey Thyroxin Into the Organ Cells

The first experimental support for the faculty of a peripheral nerve to conduct thyroxin was brought by our[129] experiments with the ischiadicus-gastrocnemius preparation of a frog.

If we put the free end of one of the two ischiadici of a frog, which are in contact with the gastrocnemius, into Ringer's solution and the central end of the other into a highly-diluted thyroxin solution (10^{-12} gm. per litre), we find, after 30–40 hours, an increase of the O_2-consumption in the muscle of the thyroxin-treated nerve of about 20–30 per cent.

We show the result of this important experiment in Table XXX.

TABLE XXX

O_2-consumption of 1.0 gm. of Gastrocnemius in 30 minutes.

Date	Ischiadicus in Ringer's solution	Ischiadicus in Thyroxin—Ringer's solution	Increase %	Remarks
7 XII 1934	50	62	24	Nerve irritable
10 XII 1934	50	66	32	,, ,,
12 XII 1934	51	67	31	,, ,,
14 XII 1934	73	88	20	,, ,,
20 XII 1934	37	46	24	3½ hrs. at 38°C.
21 XII 1934	61	77	26	Nerve irritable

In experiments, at the end of which the ischiadicus was not capable of being irritated, there was no increase of combustion, which points to the fact that passage of thyroxin takes place only in a *living* nerve.

An after-examination of this experiment by Jancso and Novak[130] confirmed our results in demonstrating an increase of combustion up to 50 per cent and, furthermore, they established that dipping of the nerve end into active concentrations of dinitrophenol could not increase combustion in the muscle cell. In this way the possible objection that, by increasing oxidation in the nerve, thyroxin perhaps provoked an irritation of the nerve which increased combustion in the muscle was ruled out.

That we have to deal here with a *passage of thyroxin* through the nerve and not with nerve irritation, is best demonstrated by the fact that the increased combustion in the muscle comes about only if the nerve is kept for at least 24 hours in the thyroxin solution, whereas after shorter periods of experimenting the oxidation remains unaltered. We can see that if we look at Table XXXI.

Particularly significant is the last experiment, No. 30, of the table, in which the nerve-muscle preparations of *two* frogs were left in the same damp chamber under the same conditions for a different length of time. After 13 hours there was no increase of combustion; after 37 hours there was an acceleration of 26 per cent.

That not only the nerve of a cold-blooded but also that of a warm-blooded animal is capable of conducting thyroxin is best demonstrated on the vagus nerve of rabbits.[131] As we showed before, a powerful increase of combustion takes place in the liver

cells 24 hours after a subcutaneous or intravenous injection of thyroxin. In order to examine the passage through the nerves of a warm-blooded animal, we infiltrated the vagus nerve of rabbits with active concentrations of thyroxin; afterwards we removed liver fragments, the first after 30 minutes, the second 24 hours later, and determined their O_2-consumption.

TABLE XXXI

O_2-consumption of whole Gastrocnemii per gm. per hour at 38°C., using Ringer's Solution or Thyroxin Solution (10^{-15} gm. per litre).

Experiment No.	Date	O_2-consumption in mm.³ per gm. per hour		Increase %	Time of Infiltration hours
		Ischiadicus in Ringer's solution	Ischiadicus in Thyroxin solution		
16	19 VI 1937	153	218	42	28
17	7 V 1937	166	192	15	40
18	7 V 1937	234	254	8	40
19	9 V 1937	123	186	51	40
20	13 V 1937	183	187	0	40
21	13 V 1937	189	227	20	40
22	14 V 1937	198	230	16	40
23	10 VI 1937	170	230	35	24
24	10 VI 1937	255	338	32	24
25	14 VI 1937	228	234	0	48
26	14 VI 1937	292	232	14	48
27	4 II 1937	159	158	0	12
28	12 II 1937	145	144	0	15
29	13 II 1937	118	112	0	15
30	10 II 1937	183	178	0	13
		153	193	26	37

The experimental arrangement, in which we did the first liver test *after* infiltration of the nerve, makes sure that the nerve damage due to infiltration does not lead to an increase of the liver oxidation. Besides this precaution, we also made control experiments: we used infiltrations with pure Ringer's solution and also active concentrations of thyroxin but removed the two liver portions within an interval of 5–12 hours.

TABLE XXXII

O_2-consumption of Liver Cells after Infiltration of Vagus, using Ringer's Solution or Thyroxin Solution (10^{-13} gm. per litre).

Date 1938	O_2-consumption mm.³ per gm. liver per hour		Difference %	Infiltrated with	Interval in hours
	1st liver portion	2nd liver portion			
22 VI	594	554	— 7	Ringer's solution	24
23 VI	613	651	+ 6	„ „	24
29 VI	694	687	— 1	„ „	24
12 X	767	729	— 5	Thyroxin solution	5
12 X	689	669	— 3	„ „	5
4 VII	646	575	—10	„ „	12
1 VII	516	618	+20	„ „	16
20 X	891	845	— 5	„ „	8
	891	1101	+23	„ „	24
21 X	828	801	— 3	„ „	8
	828	1022	+23	„ „	24

Table XXXII shows the results of these control experiments.

We see that infiltration of the vagi with pure Ringer's solution is ineffective and that the use of active thyroxin concentrations increases combustion only if at least 16 hours have passed between the endoneural injection and the second liver removal. The two last experiments in the Table, done on the same animal, are very significant; the second liver test, taken after 8 hours, showed no increase of combustion, whilst the third liver test, made 24 hours after, showed an increase of 23 per cent.

In Table XXXIII we see the main experiments in which the vagi were infiltrated with thyroxin solution (10^{-13} gm. per litre) and where the liver was tested as to its O_2-consumption 24 hours later.

TABLE XXXIII

Experiment No.	Date	O_2-consumption in mm.³ per gm. liver and per hour		Difference %
		1st liver portion	2nd liver portion	
43	30 VI 1938	590	725	+22
44	4 VII 1938	476	742	+56
45	6 VII 1938	715	785	+10
46	11 VII 1938	458	549	+20
47	20 VII 1938	597	764	+28
48	21 VII 1938	540	660	+22
49	22 VII 1938	718	736	+ 2
50	23 VII 1938	711	841	+18
51	25 VII 1938	670	762	+14
52	26 VII 1938	701	859	+22
53	30 IX 1938	646	832	+28
54	30 IX 1938	622	681	+10
55	3 X 1938	681	806	+18
56	13 X 1938	566	671	+19

In almost all of the experiments there was an increase of combustion of between 14–56 per cent, which went beyond the limit of error of the method which is about 10 per cent.

We conclude by saying that the nerves of both cold- and warm-blooded animals possess the faculty of conducting thyroxin and thus it is possible that the latter migrates into the cells via the nerves and increases combustion therein.

(*b*) Does Thyroxin Reach the Organ Cells Through the Nerves?

The experiments made on denervated kidneys, reported elsewhere, pointed to the probability that the given thyroxin enters the kidneys via the nerves. We obtained a more decisive proof, however, from the experiments in which we[131] established the time of occurrence of the thyroxin effect in the vegetative organs. These, the parotid gland, the liver, the testicles and so on, show substantial differences in proportion to their distance from the central nervous system.

If, on coming out of the blood circulation, the thyroxin indeed enters the brain first to pass thence via the nerves into the organs,

then the increase of combustion in the parotid gland must occur much earlier than in the liver, and in the latter earlier than in the testicle. To examine this, we removed from rabbits either one parotis and one testicle or liver fragments and one testicle for Warburg's experiments, injected 1 mg. thyroxin intravenously, and repeated the operation at intervals of 16–48 hours. We see the results of these experiments in Tables XXXIV and XXXV.

TABLE XXXIV

O_2-consumption of Parotis and Testicle before and after Intravenous Injection of 1 mg. Thyroxin at intervals of 16–48 hours.

xperi-ment No.	Date	Parotis				Testicle			
		O_2-consumption in mm.³ per gm. per hr.		Interval hrs.	Difference %	O_2-consumption in mm.³ per gm. per hr.		Interval hours	Difference %
		before thyroxin	after thyroxin			before thyroxin	after thyroxin		
76	17 I 1939	195	315	24	+61	234	240	24	+ 2
77	20 I 1939	239	316	24	+34	348	321	24	— 7
78	20 I 1939	220	256	24	+16	204	211	24	+ 3
79	14 II 1939	321	385	24	+20	345	341	24	— 1
80	14 II 1939	305	414	24	+36	276	304	24	+10
81	27 I 1939	232	314	36	+35	268	310	36	+15
82	27 I 1939	266	250	36	— 5	190	204	36	+ 7
83	30 I 1939	187	240	16	+28	271	263	46	— 3
84	30 I 1939	199	255	16	+28	200	275	46	+37
85	7 II 1939	264	305	16	+15	252	393	46	+56
86	7 II 1939	205	248	16	+21	195	309	46	+58

TABLE XXXV

O_2-consumption of Liver and Testicle before and after Intravenous Injection of 1 mg. Thyroxin at intervals of 16–48 hours.

xperi-ment No.	Date	Liver				Testicle			
		O_2-consumption in mm.³ per gm. per hr.		Interval hrs.	Difference %	O_2-consumption in mm.³ per gm. per hr.		Interval hours	Difference %
		before thyroxin	after thyroxin			before thyroxin	after thyroxin		
87	14 VI 1939	669	743	16	+10	284	288	24	± 0
88	14 VI 1939	688	728	16	+ 5	325	300	24	— 7
89	16 VI 1939	508	660	24	+30	330	415	48	+26
90	16 VI 1939	503	690	24	+37	310	381	48	+23

From the two series of experiments we see that whilst the testicle which is farthest away from the central nervous system shows the effect of thyroxin after 46 hours at the earliest, an increase of oxidation takes place in the liver after 24 hours and in the parotis as early as after 16 hours.

It seems certain, therefore, that when thyroxin enters the blood circulation, it is not absorbed directly by the organs but by the central nervous system; it then enters the organ cells via the nerves, where it increases combustion.

The nature of this thyroxin action would appear to be physiological. We find another kind of thyroxin effect in isolated

cells and organs when they are given thyroxin outside the body. Here damage to the cell is necessary and paralysis of the Pasteur reaction is thus effected. If this be the case, then thyroxin produces its effect directly from the blood *without latency*. We[132] are able to demonstrate that this is the case if thyroxin is administered to isolated organs or organ cells under insufficient oxygen supply. In this way it is possible to achieve a considerable increase of combustion in an isolated leg of a dog in a few minutes, as U. S. v. Euler[133] was first to demonstrate.

With these facts in mind, the question arose whether thyroxin did not exercise its effect directly from the blood—i.e., without any latent period—on the *whole* animal when the organ cells were damaged through some noxa. It seemed important to analyze this, as in a whole series of diseases, such as anaemias, uncompensated heart trouble, tumours, etc., a strong acceleration of combustion with corresponding emaciation, and even cachexia, is observed. This is difficult to explain, but may be due to the circulation of thyroxin in the blood, which in case of damage to the cells may exercise its influence directly and unhindered on the surface.

To examine this question, we anaemized white rats, partly by blood removal, partly by phenylhydrazine treatment, and tested uninterruptedly the temporal course of the effect of thyroxin in the metabolic apparatus.

It was demonstrated that as soon as the number of erythrocytes is reduced by about half, i.e., to 4–4½ millions in a rat, the administration of thyroxin leads to a powerful increase of combustion within ½–1 hour and amounts up to 50 per cent, which on a normal animal can never be observed until 24 hours have elapsed. That it was, in fact, cell damage due to an insufficient oxygen supply was demonstrated by the very interesting fact that thyroidectomized rats at a given degree of anaemia do not show the phenomenon of the immediate effect of thyroxin, because the loss of 50 per cent of the oxygen carriers does not yet signify cell damage in view of their lessened oxygen requirements and reduced sensibility with regard to lack of oxygen (Streuli, Duran.[52]) If, however, we remove from these thyroidectomized animals enough blood to reduce the number of erythrocytes to 2½–3 millions, we can see a similar effect of thyroxin without latency, as on a normal animal.[133a]

The other effect of thyroxin, the physiological, is not brought about by its circulation in the blood. As we were able to demonstrate in Chapter VI, thyroxin does not increase combustion immediately but increases the enzyme processes, and the products created thereby lead to the increase of combustion. From inside the blood, thyroxin can get only to the cell surface, where, under

normal circumstances (as Miescher[134] presumed and Warburg[135] later on proved) the oxidation takes place, which hinders the enzyme processes as a result of the Pasteur reaction. In order to be effective here, the thyroxin requires a certain damage to the cell (it need be very small) to cause the paralysis of the Pasteur reaction, as a result of which oxidation and enzyme processes run concurrently. To achieve an increase of combustion in the intact cell, thyroxin must penetrate the organ cells from the blood in a roundabout way via the nervous system. Therefore, this effect takes place only after a latency period of about 24 hours. It differs from the direct effect of thyroxin in that it shows, as Alwall demonstrated, a synergism with dinitrophenol and methylene blue. Penetrating the cell interior via the nerves, thyroxin is capable of accelerating the enzyme processes there, which secondarily leads to a furtherance of combustion. Here is the essential purpose of the passage of thyroxin through the nerves and its importance for the catalysis of cell oxidations.

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CHAPTER X

THE ROLE OF THE THYROID GLAND AND OF THE PITUITARY GLAND IN HEAT-REGULATION AGAINST COLD

Whether there exists a "chemical heat regulation", in Rubner's[136] sense, is a long-disputed question in physiology. According to its classical definition, "it comprises only those biological occurrences in which an increase of heat production is achieved in an animal at rest", i.e., where it *is brought about in organs at rest* through accelerated combustion without any discernible activity of the muscle.

The first observation to point very definitely to its existence was made by A. Montuori[137] in 1904. He reported that when normal animals were injected with the blood serum of over-heated and under-cooled dogs, the result was, in the one case, an increase of heat formation; in the other, a reduction. This report, made in Italian, remained unnoticed, and was unknown to me when, in 1912, I[138] investigated experimentally whether, in this so-called chemical heat regulation in Rubner's sense, there is substance circulating in the blood performing a function which increases combustion in the cells. I tackled this question because of the remarkable fact that the puncture of the thalamus, a single irritation of nervous centres, is followed by an increase of heat formation lasting days, which, according to the prevailing opinion of that time, came about in the peripheral organs through *increased innervation*. After all we knew regarding the reactions to irritation of nervous structures, it seemed likely that irritation of heat centres furnished only an impetus for an increased heat formation, which continued equally undisturbed also after elimination of the nervous system due, perhaps, to the hormones.

In order to prove this hypothesis, we had to examine whether increased heat formation continues to exist after elimination of the nervous system. Eight years before the description of Warburg's method, we believed that the only way to do this was to measure the sugar consumption of isolated organs of normal and feverish animals. The isolated heart of a rabbit was most suitable for the purpose because, according to the investigations made by Locke and Rosenheim,[139] it consumed an easily determinable quantity of sugar from the nutritive solution. The differences found in the sugar consumption of the hearts of normal and feverish animals

were significant: in 23 normal experiments the heart consumed per gm. and per hour an average of 2.2 mg. dextrose, whereas the hearts of 16 rabbits in a fever due to the puncture of the thalamus consumed an average of 3.8 mg. dextrose, i.e., an increased consumption of about 70 per cent.

The importance of this result lies in the fact that irritation of the heat centre also maintains a higher consumption in a peripheral organ after isolation from the nervous system. *This points to the fact that this increased consumption is not the result of increased innervation but of hormone production set in motion by puncture of the thalamus.*

Because of the world war, we were only able to resume our work again in 1919, when we examined whether hormonal factors also had a share in *physiological* heat regulation. (Mansfeld and v. Pap.[140]) These experiments, still made on isolated hearts of rabbits, afforded a further glimpse into the process of chemical heat regulation. The most essential result of these experiments was the fact that the isolated hearts of animals held in a cold or hot condition not only showed, in the first, a high sugar consumption, and, in the second, a low sugar consumption, but that the blood serum of such animals possessed the faculty of altering the sugar consumption of isolated hearts in the same way as chemical heat regulation. The serum of cooled animals increased the sugar consumption of isolated hearts, whereas the serum of warmed animals decreased it, as can be seen from Table XXXVI.

TABLE XXXVI

Sugar Consumption of Isolated Rabbit Heart per gm. and hour.

Without serum	With serum
Cooled animal	
1·50	0·69
2·28	0·94
3·60	0·41
2·80	1·22
2·92	0·97
Mean 2·62	0·84
Warmed animal	
0·85	—
1·00	—
0·80	2·57
0·27	2·82
0·58	3·56
0·80	3·46
Mean 0·71	3·10

Another discovery resulting from these experiments is the fact that this faculty of the blood sera is conditioned by the *activity of the thyroid gland,* because thyroidectomized animals furnish completely inactive blood sera despite heating or cooling.

That the thyroid gland accelerates the combustion processes has been known to us since the investigations of E. Mendel,[141] F. v. Mueller,[142] and A. Magnus-Levy.[143] But the fact that the thyroid gland intervenes in the rather complicated process of heat regulation, and supplies the blood with substances which offer protection both against too much cold and too much heat, was demonstrated for the first time by these investigations of ours.

The result of our experiments was first confirmed by the experiments of Paul Schenck[144] who was able to demonstrate that the blood of a cooled rabbit increases the O^2-consumption of a thyroidectomized receiving animal only if the donor animal is in possession of its thyroid; this harmonizes with our experiments. Our findings were further strengthened by the investigations of G. Cori,[145] made on a congenital myxoedema, which also pointed to the role played by the thyroid gland in heat regulation.

Coinciding with these experimental findings were the histological investigations of L. Adler,[146] W. Cramer,[147] and others, according to which, at a low external temperature, the thyroid glands showed the histological picture of active thyroids with high epithelium and insignificant colloid storage, whilst warm external temperature leads to an inactivation of the thyroid.

The different result of the acetonitrile reaction in summer and winter (R. Hunt[148]), as well as in warm and cold surroundings (Sundstroem[149]), pointed in the same direction; as did the investigations of L. Adler[150] regarding the awakening and heating effects of thyroid extracts on hibernating hedgehogs.

This finding was somewhat contradicted by the fact that after thyroidectomy the deficiency of the heat regulation is not of a very high degree (cf. Thauer[151]), and Hildebrandt[152] was able to demonstrate on white rats that heat production is increased in intense cold, also in thyroidectomized animals. This was obvious because the lack of the thyroid gland cannot prevent heat formation through tremor of the muscles. It led, however, to the opinion that the thyroid gland had no function with regard to the heat regulation. As Thauer[151] correctly remarks, this was completely unjustified, because one should never derive from the absence of a disturbance after removal of an organ that the latter is of no importance for the whole of the organism under physiological conditions. That, after thyroidectomy, the whole musculature may have to intervene at a temperature which normally can be mastered without tremor of the muscles, is just as possible as is the fact that animals sleeping in the open in very cold weather compensate the loss of heat, not through tremor of the muscles, but through a " hormonal " increase of heat formation. The fact that thyroidectomized animals, too, increase heat formation in cold

weather is surely no cause for denying the regulatory function of the thyroid gland in combustion. It must be remembered that we know practically next to nothing about the importance of the thyroid gland in the regulation against *over-heating,* although our above-mentioned experiments point strongly to the existence of a process, described by Plaut and Wilbrand,[153] as the "second heat regulation", and which finds expression in a decrease of combustion below the level of basal metabolism.

Doubts voiced against our experiments, however, caused us to subject the whole question of hormonal heat regulation to a renewed analysis. In the first place it was Kendall's[54] discovery that thyroxin is the active substance of the thyroid gland which increases oxidation, and the theory of its method of action described above. To-day we know that thyroxin does not display its effect on metabolism immediately, but only after a latent period of 24 hours; this was in contradiction to the fact that in our experiments the cold serum increased the sugar consumption of the heart *immediately.* The intervention of the thyroid gland in the heat balance is somewhat difficult to understand if the hormone produced by the thyroid increases the heat formation only after 24 hours.

Owing to the discovery of insulin and increased knowledge of the conditions of sugar consumption, the question arose whether it was justifiable to consider the *altered sugar consumption* of isolated hearts as the expression of *altered heat production,* as one could easily think of other processes which might effect an alteration of the sugar decrease in the nutritive solution. To analyze this seemed the more justified in view of the fact that to-day, thanks to Warburg's method, we are able to determine *combustion* instead of *the sugar consumption* in the peripheral organs. It seemed natural to examine anew *whether there appear, in heat regulation against cold, substances in the blood which accelerate or hinder combustion in the organ cells, and to investigate to what extent the endocrine organs participate in the formation of such substances.*

As symmetrical muscles furnish identical values of O_2-consumption, even if they are removed from the body after an interval of 1–2 days, it was possible, due to the described modification of Warburg's method, to examine whether, as a result of injections of blood sera of cooled animals, combustion increased in a surviving isolated muscle, i.e., after its separation from the nervous system. As test animals we used rabbits; in each case it was a *blood donor* on which, before the blood removal, cooling off with cold water and a ventilator was effected until the body temperature dropped by about 2–3° C. The animal was then dried

and the ventilator switched off. When the body temperature rose again by about 0.5–1.0° C. blood was removed from the animal for the preparation of the serum. The sera thus obtained were injected into one to three *blood receivers* intravenously in quantities of 5 c.c. for each experiment. Directly before the injection one gastrocnemius was cut out from the blood receivers under asepsis, any bleeding was carefully stopped, and the wounds stitched up. The gastrocnemii were immediately weighed in sterile weighing tubes, suspended in the sterile Warburg vessels, and their O_2-consumption was determined in the 2-hour experiment. The second gastrocnemius was removed in the same way 20–30 minutes after the serum injection and then proceeded with.

We referred earlier to the contradiction which exists between the results of our former experiments and the mode of action of thyroxin which is now recognized. Whilst the tested sera of freezing animals (which we shall call " cold sera ") were *immediately* active in the muscle cells of the heart, we now know that thyroxin increases combustion only after a latent period of 24 hours, and, as we have demonstrated earlier, this increase takes place in the vegetative organs and not in the muscle cells themselves.

In the first place we had to investigate whether the cold serum leads immediately to an increase of the O_2-consumption; further, whether this increase—similarly to thyroxin—occurs in the vegetative organs or in skeletal muscle.

TABLE XXXVII

Action of 5 c.c. of Blood Serum of cooled Animal on O_2-consumption of Muscle and Liver of Blood Receiver.

Experiment No.	Date 1938	Blood donor	Blood receiver	O_2-consumption in mm.³ per gm. per hour					
				Muscle			Liver		
				Before serum	30 mins. after injection	Difference %	Before serum	30 mins. after injection	Difference %
1	11 XI	A	1	207	337	+62	631	617	—2
2	14 XI	B	2	180	246	+36	559	545	—3
3	25 XI	C	3	186	286	+54			
			4	213	224	+ 5			
4	30 XI	D	5	203	266	+31	607	549	—9

For this purpose, both liver and muscle fragments were removed from the blood receivers before and after the injection of serum in the way described:[153a]

An abdominal incision 2–3 cm. long is made under strict asepsis, and by means of a conchotome used in rhinotomy 4–5 small round liver pieces of about 0.12 gm. are cut out from the various liver lobes. The bleeding of the liver is insignificant and stops almost instantly.

The result of these experiments is compiled in Table XXXVII.

We see that a very considerable increase of combustion took place in the isolated muscle, which we removed 30 minutes after the administration of the cold sera, whereas combustion in the liver cell remained unaffected by the serum.

The immediate efficacy of the serum as well as its localization spoke decisively against the assumption that the acceleration of combustion is brought about through thyroxin. It had to be examined whether the thyroid gland is necessary for the formation of effective cold sera.

To analyze this we used *thyroidectomized animals as blood donors.* The sera obtained after the cooling process were injected into normal blood receivers, and combustion in the muscle was determined. We see the result in Table XXXVIII.

TABLE XXXVIII

Action of 5 cc. Blood Serum of cooled Thyroidectomized Animal on O_2-consumption of Muscle.

Experiment No.	Date 1938	Blood donor (thyroidectomized)	Blood receiver	O_2-consumption mm.³ per gm. muscle per hr.		
				Before serum injection	30 mins. after serum injection	Difference %
5	28 XI	E	6	237	239	0
			7	263	262	0
			8	264	234	—11
6	29 XI	F	9	221	234	+ 5
			10	242	207	—10
			11	251	293	+16
7	9 XII	G	12	231	253	+13
			13	309	292	— 5
			14	234	239	+ 2

The cold serum of three thyroidectomized animals proved to be completely ineffective on seven receiver-animals, whereas the insignificant increase of about 13 per cent on one and 16 per cent on another scarcely surpasses the limit of error.

There can, therefore, be no doubt whatsoever that the activity of the thyroid gland is needed for the formation of effective cold sera.

The experiments that follow show the extent and the manner of the participation of thyroxin in this activity.

The fact that the thyroid gland is necessary for the formation of effective cold sera, and that its active substance alone is incapable of accelerating combustion in the muscle, points to the probability that, in the regulation against cold, thyroxin is supplied to the blood. *It does not, however, increase combustion in the muscle directly, but causes the formation of a second active substance.*

To test the correctness of this assumption, the blood donors

were injected with 0.5 mg. thyroxin instead of being cooled, and blood was taken after an hour; the " thyroxin sera " thus obtained were administered to normal blood receivers.

The result is shown in Table XXXIX.

The injection of thyroxin into the blood has, as we see, the same effect as the regulation against cold. In both cases substances get into the blood which increase combustion in the isolated muscle when transferred to other animals. Hence it follows that the thyroid gland is absolutely necessary for the cold sera to become effective. As a result of the influence of cold, the thyroid delivers

TABLE XXXIX

Action of " Thyroxin Sera " on O_2-consumption of Muscle of Normal Blood Donor.

Experiment No.	Date 1939	Blood donor (0·5 mg. thyroxin intravenously)	Blood receiver (normal)	O_2-consumption mm.³ per gm. muscle per hr.		
				Before serum injection	30 mins. after serum injection	Difference %
8	17 III	H	15	216	293	+35
			16	186	273	+46
			17	128	267	+108
9	18 III	I	18	233	280	+20
			19	159	293	+84
			20	176	262	+48

thyroxin which, in its turn, causes the formation of yet another (second) active substance; this latter substance can be transferred with the serum and immediately accelerates combustion in the muscle. Our next task was to explore the origin of this active substance.

It was certain that the mobilization of the actual active substance of the cold sera through thyroxin occurs in one of the endocrine glands which, according to earlier investigation, intervene in the heat regulation. In the first place we thought of the suprarenal gland which, according to Cannon[154] and Geiger,[155] plays a role in the regulation against cold, and, secondly, of the hypophysis, whose importance for heat regulation has been under discussion ever since the investigations made by Hashimoto[156] and Kuschinsky.[157]

We therefore investigated whether the formation of effective cold sera is prevented by elimination of these organs.

For this purpose we ligatured both suprarenal glands in the blood donors, waited ½–1 hour until they recovered from the operation—which lasted only a few minutes—and injected 0.5 mg. thyroxin intravenously. An hour later they were bled to death, and the serum was injected into normal animals.

The removal of the hypophysis was done under light narcosis with ether. Penetration was along the throat laterally from the

tongue bone. The hypophysis was laid free by boring into the base of the skull and removed by means of a vacuum pump. After the operation the animals spent one night in the heat box, recovered very quickly, showed (contrary to Hashimoto's[156] findings) normal body temperature on the very next day, and were in a good condition generally. One to two days after the operation they were used for the serum preparations. Blood removal took place one hour after the injection of 0.5 mg. thyroxin. Table XL shows the result.

TABLE XL

Effect of "Thyroxin Sera" of Animals without Suprarenal Gland or Hypophysis on O_2-consumption of Muscle of Normal Blood Receiver.

Experiment No.	Date 1939	Blood donor (0·5 mg. thyroxin intravenously)	Blood receiver (normal)	O_2-consumption mm.³ per gm. muscle per hr.		
				Before serum injection	30 mins. after serum injection	Difference %
10	22 III	K	21	186	211	+17
			22	89	109	+22
11	23 III	L	23	210	207	± 0
			24	179	228	+27
12	24 III	M	25	258	262	± 0
			26	191	253	+33
13	27 III	N	27	221	196	—11
			28	236	223	— 5
14	28 III	O	29	243	267	+ 9
			30	236	241	+ 2

K, L, M without suprarenal gland.
N. O without hypophysis.

We see that whilst elimination of the suprarenal gland was irrelevant as far as the formation of the active substance of the serum was concerned, *animals without their hypophysis lost the faculty of producing effective sera on administration of thyroxin.*

TABLE XLI

Effect of Blood Serum of cooled Animals (without Hypophysis) on O_2-consumption of Muscle of Normal Blood Receiver.

Experiment No.	Date 1939	Blood donor (without hypophysis)	Blood receiver	O_2-consumption mm.³ per gm. muscle per hr.		
				Before serum injection	After serum injection	Difference %
33	28 III	P	31	237	245	+ 3
			32	211	214	± 0
34	29 III	Q	33	294	286	— 3
			34	250	261	+ 4

There was also the question whether the influence of cold required the hypophysis in order to produce effective cold sera.

This we tested on hypophysectomized blood donors, exposed to the effect of cold in the usual way. These experiments are classified in Table XLI.

It was demonstrated that hypophysectomized animals, when exposed to cold, give ineffective sera in the same way as in thyroidectomized ones (see Table XXXVIII), so that co-operation between thyroid gland and hypophysis is necessary for the formation of the combustion-increasing substance—*the so-called heating hormone.* The procedure is the following: *In the regulation against cold the thyroid gland supplies the blood with thyroxin; the latter leads, with the aid of the hypophysis, to the formation of the active substance which is humorally transferable and which immediately increases combustion in the muscle of normal animals.*

The question arose further whether thyroxin, after its injection into the blood, increased combustion without latency in the muscles of the same animals; in all our former experiments we usually investigated the effect of thyroxin on the muscles only after a latent period of 24 hours, and found it always ineffective.

To examine this, we injected thyroxin into the blood of normal and hypophysectomized rabbits, and determined their O_2-consumption in the isolated gastrocnemius 1–3 hours later.

We see the result in Table XLII.

TABLE XLII

O_2-consumption of Isolated Gastrocnemius before and 1–3 hours after Thyroxin Injections in Normal and Hypophysectomized Rabbits.

Date 1941	O_2-consumption mm.³ per gm. muscle per hour		Difference %	Interval after thyroid injection hours
	Before thyroxin	After injection		
Normal animals				
8 III	253	320	+26	1
8 III	187	223	+16	1
8 III	160	248	+54	1
8 III	246	319	+30	1
10 III	248	289	+16	1
10 III	214	260	+21	1
10 III	206	236	+14	1
10 III	173	208	+20	1
14 III	216	293	+35	3
14 III	225	293	+25	3
27 III	236	345	+45	3
27 III	193	333	+72	3
28 III	176	240	+35	3
28 III	157	211	+34	3
Hypophysectomized animals				
17 III	241	264	+ 9	2
17 III	195	188	— 3	2
17 III	292	297	± 0	1
17 III	255	227	—11	1
28 III	209	199	— 5	3
28 III	166	156	— 6	3

In fact thyroxin leads shortly after its incorporation to an increase of combustion in the resting muscle, but it takes place only in those animals which are in possession of their hypophysis.

As a result of these experiments we discovered a hitherto unknown effect of thyroxin, which causes the hypophysis to deliver

to the blood an active substance, which immediately increases combustion in the resting muscles and thus intervenes in the process of chemical heat regulation.

The old disputed question, whether there can be an increase of combustion without discernible activity of the muscles, has now been decided positively, because we see that in the cold a substance is formed which increases combustion in the resting muscle and makes possible an increase in heat formation without tremor of the muscles. This must be of importance for the endurance of temperature below the comfort limit, because this substance postpones the unpleasant "freezing" as long as possible, in order to protect us from suffering. This seems to be an important task of hormonal heat regulation, which perhaps attains its real importance in animals sleeping in the open at a low external temperature by preventing *tremor of the muscles,* which is incompatible with sleep.

In the chemical heat regulation thyroxin has to perform yet another function. Having entered the blood, it leads 24 hours later to a promotion of heat formation in the vegetative organs lasting many days. In the regulation against cold we have seen that thyroxin is delivered to the blood, and therefore it is to be expected that every cooling-off of the body should bring about an increase of combustion in the inner organs after a latent period of 24 hours. That this is really the case was demonstrated on rabbits; liver fragments were removed from them before the cooling-off of the body and also 24 hours later, and then tested as to their O_2-consumption.

The result is to be seen in Table XLIII.

TABLE XLIII

O_2-consumption in Liver Cells of Rabbits before and 24 hours after cooling.

Date 1940	O_2-consumption mm.³ per gm. liver per hour		
	Before cooling	24 hours later	Difference %
9 II	546·8	741·9	+36
9 II	473·2	661·9	+40
10 II	448·3	651·9	+45
10 II	566·0	745·2	+32

This belated effect of thyroxin may well be of importance for adaptation to cold surroundings, because heat formation increased in the inner organs due to thyroxin, i.e., without participation of the muscles, is not felt and independent of the unpleasant feeling of cold. Finally, the secreted thyroxin has yet another role to play in regulation against cold: Chachovitsch[92] demonstrated

that the *efficacy of the tremor of the muscles* for heat formation, i.e., the calorific effect of the muscle-contraction, is strengthened by thyroxin, so that the secretion of thyroxin in the cold has a favourable effect, even when it can no longer prevent the tremor of the muscles, as in excessive cold.

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CHAPTER XI

SEASONAL CHANGES IN THE SENSITIVITY OF WARM-BLOODED ANIMALS TO THYROXIN

THE *experiments* reported in Chapter VI, concerning the effect of thyroxin on surviving tissues, show the remarkable fact that spring is not a suitable season for bringing about the oxidative effect of thyroxin on surviving cells. A similar seasonal failure of the thyroxin effect can be seen in the frog,[75] in which there is no increase of the protein metabolism through thyroxin in the winter, but which asserts itself in full strength in the warm seasons.

This seasonal vacillation of the sensitivity to thyroxin was further confirmed by another observation, which we must discuss fully, as it proved to be decisive for our investigation of the physiology of the thyroid gland.

The antagonistic effect of thyroxin with regard to the effect of novocaine on temperature has already been fully discussed in Chapter VIII. If we inject a guinea-pig according to Glaubach and Pick[103] with 0.2 gm./kg. novocaine, there occurs a fall of temperature of 3–4°C. If this novocaine experiment, however, is made after a preliminary treatment with thyroxin, then the novocaine has almost no influence on the body temperature. That both novocaine and the contrary effect of thyroxin influence the *loss of heat* but not the production of it could be demonstrated either by our metabolic experiments or through thermoelectrical measurement of the loss of heat. This effect of thyroxin on the loss of heat proved to be a central one, because it can be prevented through intersection of the spinal cord (D 5–D 8) or through ergotamine. If the novocaine experiment is executed on an animal whose spinal cord had been intersected or on an ergotaminized one, then thyroxin can do nothing to prevent the fall of temperature brought about by novocaine.

This phenomenon was not fully acceptable, because it is known that in addition to this central effect on the loss of heat, thyroxin increases combustion, and that this effect on heat formation also remains unaltered after intersection of the spinal cord. (Oberdisse and Roda.[97]) Why, it must be asked, is the increased loss of heat after novocaine not compensated through the oxidative influence of the thyroxin? An exact investigation of these conditions revealed a most interesting "seasonal change" of the thyroxin effect. It was demonstrated that the thyroxin-novocaine

antagonism through intersection of the spinal cord or ergotamine is prevented only in the warm season, i.e., from March until November, while in the winter thyroxin remains effective on such animals and works against the novocaine through increasing combustion. It was also shown that, whilst in winter the applied dosages of thyroxin provoke a powerful increase of combustion (35 per cent), in the warm season similar quantities have no effect on metabolism and triple quantities must be administered in order to increase combustion.

We show in Table XLIV that in the spring thyroxin becomes ineffective with regard to combustion where experiments are given in which we determined the CO_2-production of guinea-pigs in February and May according to the method of Oberdisse and Roda.[97]

TABLE XLIV

Date 1937	Animal No.	Weight of Animal, gm.	CO_2-production mg. per gm. per hour		Difference %	Dose of thyroxin mg. per 100 gm. animal
			Normal	After 3 × 0.3 mg. thyroxin		
February	1	424	1·35	1·86	37·7	0·21
	2	410	1·47	1·94	32	0·21
May	3	478	1·65	1·82	10	0·19
	4	405	1·67	1·85	10	0·22
	5	540	1·73	1·91	10	0·17

It was thus demonstrated that in warm seasons, contrary to general belief, it is not the production of thyroxin which is diminished, but its *efficacy which is reduced,* as we have already seen on surviving organs.*

The fact that the novocaine-antagonistic effect of thyroxin disappears through intersection of the spinal cord only in the warm season, and that in the winter it prevents fall of temperature on animals whose spinal cord has been intersected, through a peripheral increase of combustion, gave us a simple method of determining the varying sensitivity of animals towards thyroxin without having to resort to any metabolic experiments.

This " test of the effect of thyroxin " was done on animals whose spinal cord had been intersected according to Glaubach and

* The contrary experiment made by Issekutz (*Arch. exp. Path. Pharm.* (1942), **200**, 156) that, in winter, there can be no increased effect of thyroxin on combustion, is easily explained. Unfortunately he always determined the O_2-consumption only, which is certainly more convenient, but which may lead to uncontrollable errors when we deal with the effects of hormones on metabolism. We found that in winter administration of thyroxin not only increases combustion but also increases the R.Q., so that part of the consumed oxygen is derived from glycogen and escapes determination.

Pick by analyzing the effect of novocaine on the body temperature before and after thyroxin treatment (3 × 0.3 mg.). The undiminished effectiveness of thyroxin on combustion is demonstrated by the fact that no fall of temperature due to novocaine takes place in the animal despite the intersection of the spinal cord, because thyroxin, by increasing heat *production*, counteracts the increased *loss* of heat. However, if there is a diminished sensitivity of the animals towards thyroxin—this is the case in the warm season—then preliminary treatment with thyroxin cannot prevent the above-mentioned fall of temperature, conditioned by novocaine.

We show in Diagram 29 the varying efficacy of thyroxin in winter and spring.

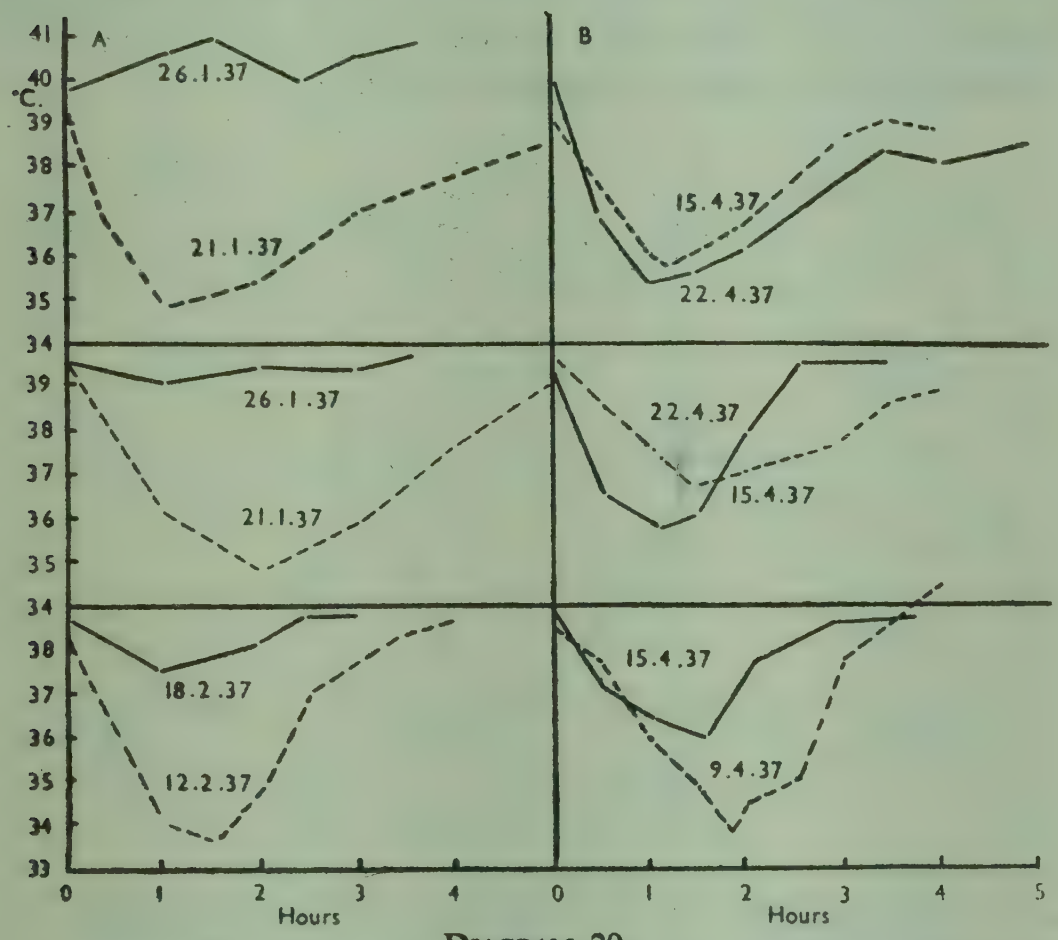


DIAGRAM 29.

Novocaine-antagonistic thyroxin effect on guinea pigs with their spinal cords dissected at D 5–6.

A. Winter. B. Spring.

- - - - Novocaine effect before thyroxin treatment.

—— Novocaine effect after thyroxin treatment.

Ordinate: Body temperature.

In this way we came into possession of a relatively simple and most reliable test, which makes it possible to pursue the vacillations of the sensitivity to thyroxin as well as to investigate their causes.

CHAPTER XII

THE CAUSE OF DIMINISHED SENSITIVITY TO THYROXIN IN THE WARM SEASON

OUR assumption was that with the coming of spring an active substance was produced through some endocrine gland which, due to its antagonistic effect, lowered the efficacy of the thyroxin. In the first instance we thought of the gonads, and therefore tested the efficacy of thyroxin on normal and castrated guinea-pigs *in summer*. If the gonads were able to hinder the effect of thyroxin through an active substance, thyroxin ought to prevent a fall of temperature in castrated animals in summer.

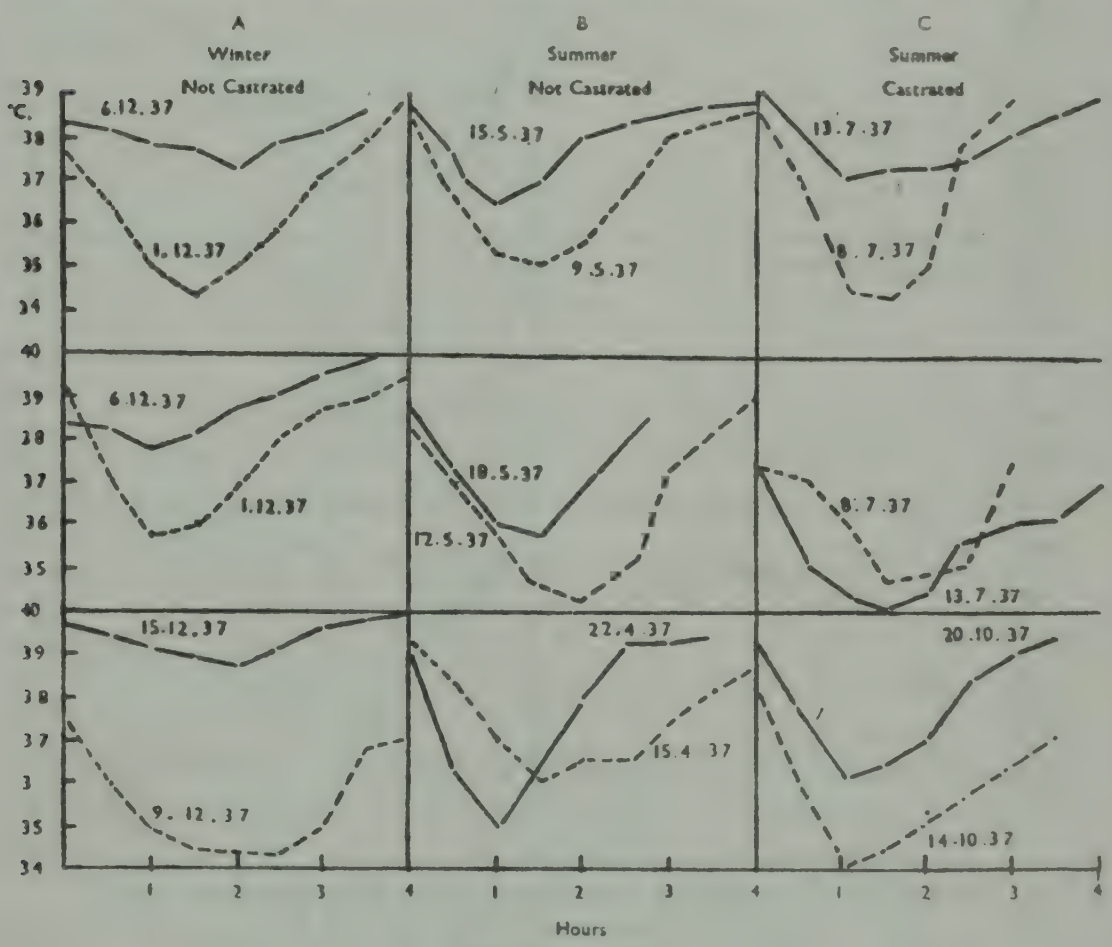


DIAGRAM 30.
Novocaine-antagonistic thyroxin effect on guinea pigs with their spinal cord dissected at D 5–6.
- - - - Novocaine-effect before thyroxin-treatment.
—— Novocaine-effect after thyroxin-treatment.

However, we see in Diagram 30 that this was not the case, because even in castrated animals the fall of temperature, due to novocaine, took place undiminished despite preliminary treatment with thyroxin.

It was thus demonstrated that the lowered efficacy of thyroxin, which sets in in the spring, does not depend on the activity of the gonads.

On the other hand, a most interesting fact to be established was that *the thyroid gland itself lowers the efficacy of the thyroxin in the warm season, as we shall see, through secretion of an active substance.*

If we analyze the efficacy of thyroxin in summer on *normal* and *thyroidectomized* animals whose spinal cord has been intersected, we see that the thyroidectomized animals behave in summer in the same way as winter animals: despite intersection of the spinal cord, thyroxin prevents the fall of temperature. We see in Diagram 31 how thyroxin regains its efficacy in summer after thyroidectomy.

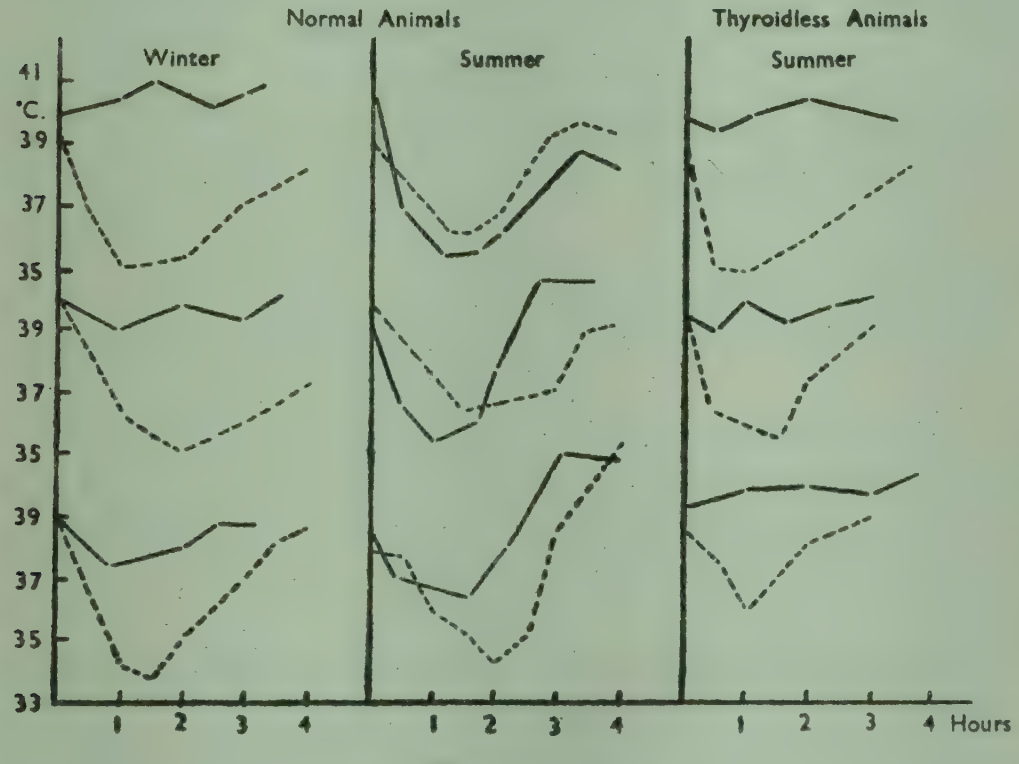


DIAGRAM 31.

Novocaine-antagonistic thyroxin effect in winter, its disappearance in summer and its restoration by thyroidectomy.

---- Novocaine effect before thyroxin treatment.

—— Novocaine effect after thyroxin treatment.

The seasonal vacillation of thyroid activity, often maintained on the clinical side, was thus shown to be contrary to expectations, an elimination of the over-large production of heat in the spring, not through lowering the *production of thyroxin,* but by rendering *the cells of the organs insensitive to its oxidation effect.* The probable reason for this arrangement may be found in the fact that when the warm season comes the organism can do with-

out the heating effect of thyroxin, but cannot do without the other effects of this hormone, which may well be of particular importance in the spring. Significant in this respect is our finding[75] that a frog's organism becomes refractory to thyroxin not in the spring, but during the winter, i.e., at a time when the frog must use its stores sparingly, but at the same time has to produce the ova for which thyroxin may be necessary.

In full accordance with this is the work of M. G. Sax and R. G. Leitson[158] in which is demonstrated the indispensability of thyroxin for the development of the fertilised ovum in a rabbit.

We shall report about our further investigations to clarify this new effect of the thyroid gland later on.

158. Sax, M. G., and Leitson, R. G.: *Bull. Bid. et Med. exp.* IV, (1938), p. 496.

PART THREE

THE THERMOTHYRINS

CHAPTER XIII

THE DISCOVERY OF THE THYROXIN-ANTAGONISTIC PRINCIPLE OF THE THYROID GLAND

FROM experiments reported in the previous chapter we drew the conclusion that, with the coming of the warm season, the thyroid gland begins to produce an active substance which works against the oxidation-increasing effect of thyroxin and thus lowers its efficacy. *Our next task was to find this thyroxin-antagonistic element in the thyroid gland.*

For these investigations we had only the three winter months of December, January, and February at our disposal, so that our work was protracted for four years before we reached our goal. The analysis proceeded as follows:

In the winter months we subjected guinea-pigs whose spinal cord had been intersected to a preliminary treatment with the compound to be examined. We administered these substances to the animals 3–6 days before treatment with thyroxin as well as during the 3 days of treatment with thyroxin, and examined the effect of novocaine on them. This was in order to find out whether the administered substance reduces the efficacy of thyroxin to the same extent as the anticipated active substance of the thyroid gland in summer. Each of these principal experiments was preceded by a preliminary experiment, in which the effect of novocaine alone was tested, and, parallel to these investigations, we always determined the efficacy of thyroxin in the novocaine experiment on a few control cases without any preliminary treatment.

At first we tested the 3 : 5 di-iodotyrosine which for some years was reputed to be an antagonist of thyroxin,[159] and then iodothyreopeptone which, according to Abelin[160] who prepared it, works against the metabolic effect of thyroxin. Three days before and for 3 days during the treatment with thyroxin the animals received subcutaneously each time 10 mg. di-iodotyrosine. We

administered the iodothyreopeptone according to Abelin's prescription enterally in dosages of 10 mg. 6 days before and for 3 days during the treatment with thyroxin. In Diagram 32 we show three examples in which we tried to prevent the effect of thyroxin with these substances.

Both substances proved to be completely ineffective, because the thyroxin became fully active and prevented the diminution of temperature by novocaine in animals with intersected spinal cord, as is the rule in winter.

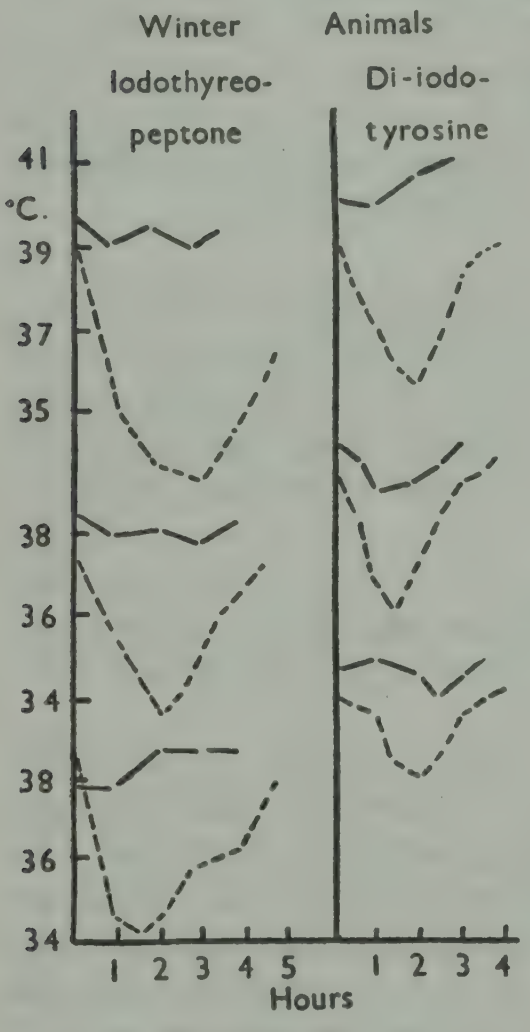


DIAGRAM 32.
Novocaine-antagonistic thyroxin effect after pretreatment with iodothyreopeptone and di-iodotyrosine.
- - - - Novocaine effect before thyroxin treatment.
—— Novocaine effect after thyroxin treatment.

Therefore neither di-iodotyrosine nor iodothyreopeptone posseses the faculty of substituting the function of the thyroid gland which hinders the oxidative influence of thyroxin with the coming of spring.

After this failure we tried to produce various fractions from hydrolyzed thyroid glands. We carried on with these experiments during the winter months of 1937–38 and 1938–39 without success. Finally, during the winter of 1939–40, we succeeded in obtaining from the hydrolysate of the thyroid gland, after removal of the thyroxin, an extract which possesses the faculty of transforming "winter animals" into "summer animals", so that animals which have had preliminary treatment with this extract display in the winter the same diminished sensitivity to thyroxin as summer animals.

To obtain such effective extracts, thyroid glands of cattle, dried *in vacuo*, are hydrolyzed for 6 hours with a 10 per cent $Ba(OH)_2 + 8H_2O$ (Merck) according to Romeis[161] and Harington[162] in order to isolate the thyroxin, and filtered; the filtrate is precipitated with HCl (precipitate A); the insoluble part is freed from Ba through washing with HCl, dried and extracted with ether (precipitate B). A and B are united and hydrolyzed with 40 per

cent $Ba(OH)_2 + 8H_2O$ for 18 hours and filtered; the filtrate is then precipitated with HCl (precipitate C). The insoluble Ba salts are completely freed from Ba through boiling with 10 per cent Na_2SO_4 solution (i.e. until the filtrate does not give any more precipitate with alkaline Na_2SO_4 solution) and filtered (filtrate A). Acid precipitate C is dissolved in N/1 NaOH and united with filtrate A. These alkaline solutions are now brought with H_2SO_4 to pH5 and left in the refrigerator for 2×24 hours. A white precipitate formed, which contains the thyroxin, is filtered; the filtrate is precipitated with abs. alcohol (96 per cent), and the alcohol is distilled off *in vacuo*. After removal of the alcohol a precipitate is formed, which is dissolved by addition of N/1 NaOH. The slightly alkaline, faintly opalescent, solution is distilled down to the desired volume, filled into phials, and sterilized in steam.

In our experiments on guinea-pigs whose spinal cord had been intersected, about which we shall presently report, we used a solution which corresponded to 1 gm. per c.c. of *fresh* thyroid. The animals received 1 c.c. on each occasion three days before and for three days during the treatment with thyroxin. In the first column of Diagram 33 we see the

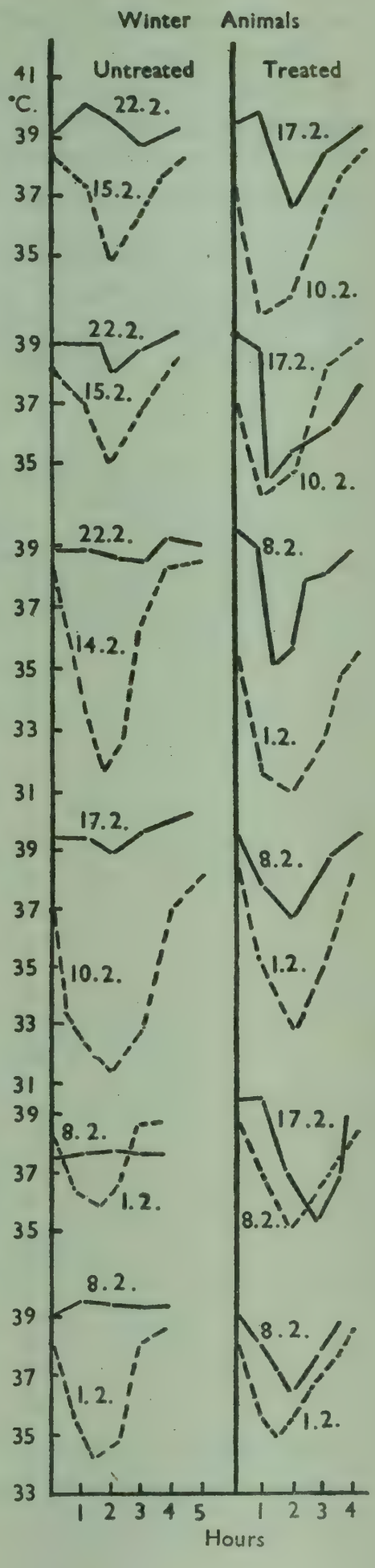


DIAGRAM 33.

Prevention of novocaine-antagonistic thyroxin effect by pretreatment with the new thyroid hormone, " thermothyrin ".

- - - - Novocaine effect before treatment.
—— Novocaine effect after treatment.

effect of thyroxin on animals which had not been treated, in the second its effect on animals with a preliminary treatment.

The experiments demonstrate that the new active substance of the thyroid gland prevented the novocaine-antagonistic effect of thyroxin in all the experiments made, and that animals which have had a preliminary treatment with it behave in the same way as after the arrival of spring.

Thus we succeeded in producing from the thyroid gland an active substance which is capable of substituting in winter animals the thyroxin-antagonistic function of the thyroid gland which sets in in the spring. In the future we shall call it thermothyrin.

159. ABELIN AND SCHÖNENBERGER: *Z. ges. exp. Med.* (1933), **90**, 489.
160. ABELIN: *Biochem. Z.* (1936), **286** 160.
161. ROMEIS, B.: *Biochem. Z.* (1922), **133**, 97.
162. HARINGTON, C. R.: *Fortischritte der Chemie organ., Naturstoffe.* Vol. II. (1939 , p. 103. *The Thyroid Gland: its chemistry and physiology*. Oxford University Press, 1933.

CHAPTER XIV

THE EFFECT OF THERMOTHYRINS ON COMBUSTION

It goes without saying that we could not be satisfied with the discovery that the newly-recognized active principle of the thyroid gland diminishes the efficacy of thyroxin in the novocaine experiment; we had, of course, to analyze its effect on combustion from two angles. Firstly, does thermothyrin annihilate the oxidation-increasing effect of thyroxin? Secondly, it is then capable of lowering the basal metabolism of normal animals?

(*a*) Antagonistic Effect Towards Thyroxin

Experience acquired over many years teaches us that the surest way of demonstrating the oxidation-increasing effect of thyroxin is on rabbit's liver. Dresel[77] was the first to show that preliminary treatment with thyroxin leads to an increase of the O_2-consumption in isolated liver cells. We use his experiment for proving the effect of thyroxin in the following way. We remove (as already described) small liver fragments from the same animal before the thyroxin injection and again 24 hours later, and determine their O_2-consumption after Warburg's method. This thyroxin test always works and is not subject to seasonal vacillations, provided we use sufficiently large doses. We give intravenously 1 mg. thyroxin per rabbit.

Table XLV

Experiment No.	O_2-consumption mm³. per gm. liver per hour		
	before thyroxin	24 hours after thyroxin	Difference %
1	569	742	+30
2	541	752	+39
3	550	684	+24
4	595	720	+21
5	508	723	+42
6	489	607	+24
	Thermothyrin intravenously (1 hour before second liver removal)		
7	566	639	+13
8	662	705	+ 6
9	695	752	+ 8
10	604	639	+ 6
11	476	519	+ 8
12	689	653	— 6

In Table XLV we show this effect of thyroxin on normal rabbits and on rabbits which had had preliminary treatment with thermothyrin. Thermothyrin was administered intravenously 1 hour before

the second liver removal, i.e., 23 hours after the thyroxin, in order to see *whether it antagonizes the already developed effect of thyroxin.*

In order to convince ourselves of the efficacy of the thyroxin, on two occasions we did the second liver removal 24 hours after the thyroxin injection; then we injected thermothyrin and repeated the liver removal 1 hour later.

DIAGRAM 34.

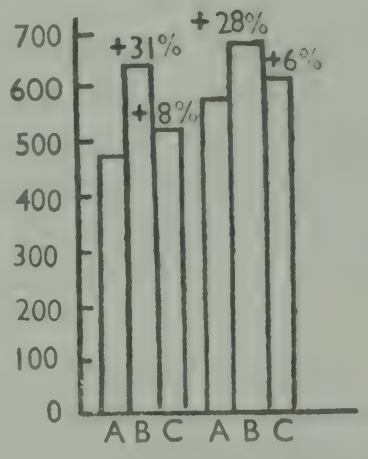
Destruction of thyroxin effect on the liver of rabbits by thermothyrin.

A. Normal O_2-consumption of liver cells.

B. 24 hours after 1 mg. thyroxin.

C. 1 hour after thermothyrin.

Ordinate: mm.^{3} O_2-consumption per hour per gm. liver.



These experiments, shown in Diagram 34, demonstrate beautifully how thermothyrin annihilates the effect of thyroxin within 1 hour.

DIAGRAM 35.

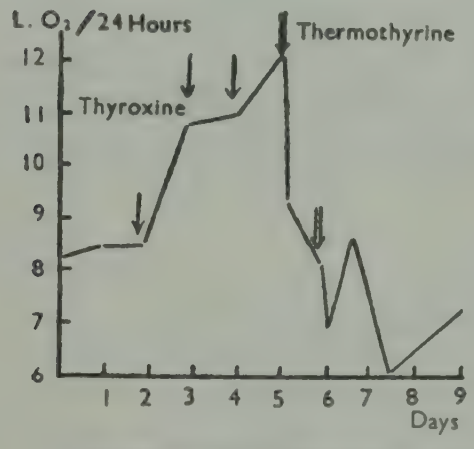


DIAGRAM 36.

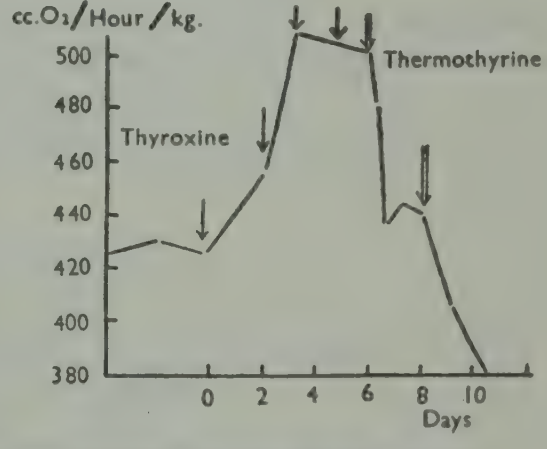


DIAGRAM 35. Thyroxin-antagonistic action of thermothyrin on rat.

↓ 3 × 0.3 mg. thyroxin.

⇓ 2 × 2 c.c. thermothyrin.

DIAGRAM 36. Thyroxin-antagonistic effect of thermothyrin on thyroidectomized dog.

↓ 4 × 5 mg. thyroxin.

⇓ 2 × 5 c.c. thermothyrin.

We then analyzed the thyroxin-antagonistic effect on white rats and dogs whose O_2-consumption was caused to rise through administration of thyroxin.

The experiments on rats were made with the apparatus of Belak and Illenyi.[163] The O_2-consumption of the dogs was determined after Krogh.

We see the results of these experiments in Diagrams 35 and 36.

Diagram 35 demonstrates an experiment on a rat of 330 gm. Administration of 3×0.3 mg. thyroxin increased the O_2-consumption by 41 per cent. After the supply of 2×2 c.c. thermothyrin, corresponding to 4 gm. fresh thyroid, the O_2-consumption sank by 49 per cent and was 28 per cent lower than the initial value.

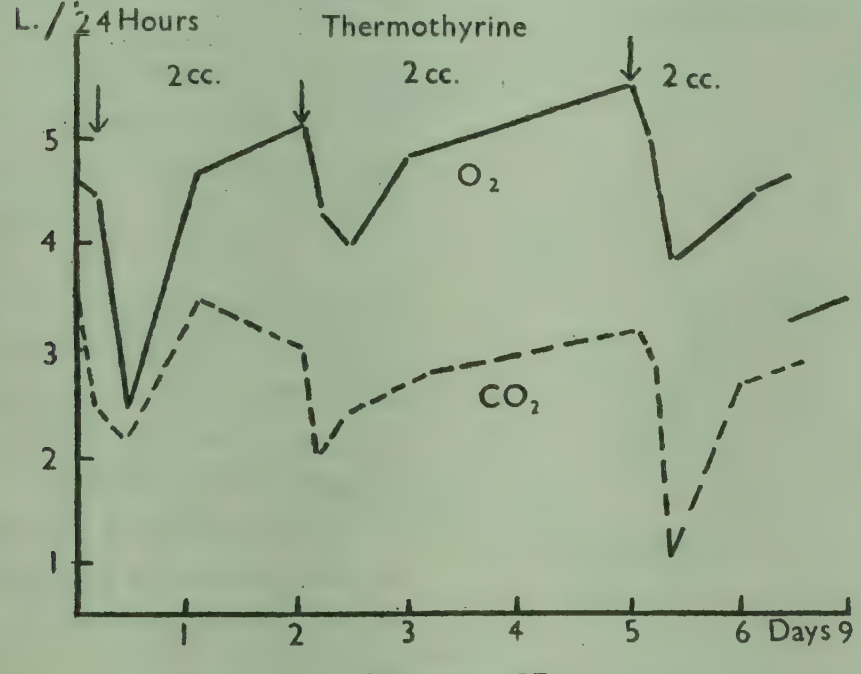


DIAGRAM 37.
Effect of thermothyrin on the gas-exchange of a rat.

Diagram 36 shows an experiment on a thyroidectomized dog of 12 kg. whose O_2-consumption was raised by about 30 per cent in four days through administration of thyroxin. Two injections of thermothyrin of 5 c.c. each led to a fall of 47 per cent, and the O_2-consumption remained about 30 per cent below the original value.

The effect on both kinds of animals was alike: *the O_2-consumption, increased through thyroxin, fell considerably below normal.*

(*b*) Effect on the Basal Metabolism

The experiments on animals treated with thyroxin demonstrated that thermothyrin not only annihilates the increase of the O_2-consumption, but lowers it well below normal if applied in suffi-

ciently large quantities. This made us presume that thermothyrin acts not only as an antagonist with regard to thyroxin but that it also reduces the basal metabolism. In order to examine this, we investigated its effect on gas exchange in normal rats and in curarized dogs during the acute experiment and show the result in Diagrams 37 and 38.

In both kinds of animals we find a very considerable hindrance of the combustion which sets in immediately after the injection.

When we started the purification of this principle of the thyroid gland with its oxidation-hindering and thyroxin-antagonistic effect, we discovered that the extracts tested by us *contained two substances which were active on metabolism*, both lowering combustion. The solution used in the above experiments is slightly alkaline. If it is treated with HCl, we obtain a *precipitate* which is easily soluble in aqueous N/10 NaOH.

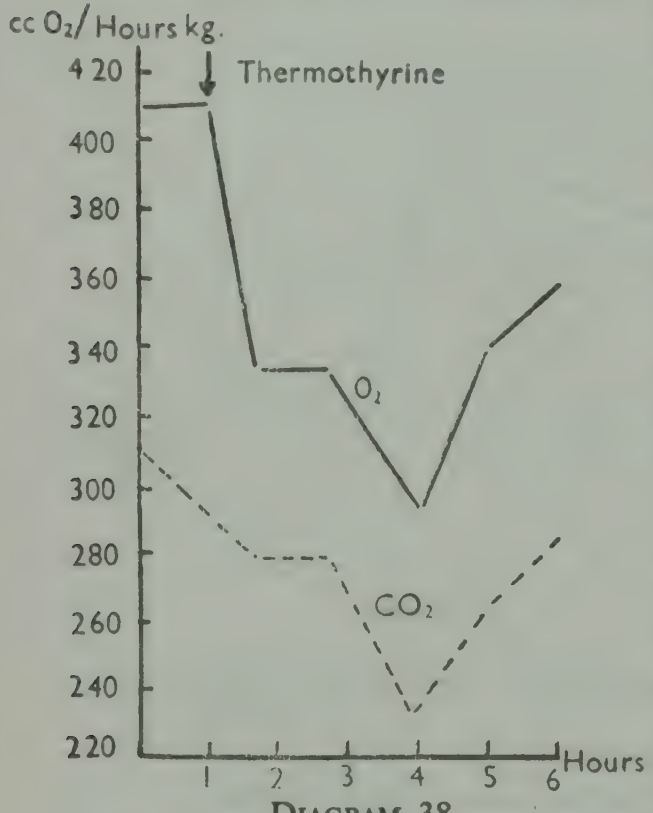


DIAGRAM 38.
Effect of thermothyrin on the gas-exchange of a curarized dog.

An analysis of the *filtrate* in the metabolic experiment, after separation from the acid precipitate and neutralization, demonstrated that the effect on combustion is not lost through the acid precipitation. When we finally analyzed the acid precipitate after purification with alcohol in an alkaline solution, we discovered that this substance, too, lowered combustion.

We show the effect of both these substances on rats in Diagram 39.

We call the acid-soluble active substance *thermothyrin A*, the acid-insoluble *thermothyrin B*.

We shall report more about the further characteristics of these substances later. Before dealing more fully with their physiological importance, however, we must mention the development of those experiments which were devoted to the question of the hormonal factors of " chemical heat regulation ". These investigations were concerned with the problem of whether in the *regulation of the body*

temperature against heat, too, active substances get into the blood which influence combustion by chemical means, as we saw in the regulation of the body temperature against cold. They led us to recognize active substances hindering oxidation, which proved to be identical with the thermothyrins which we have just described.

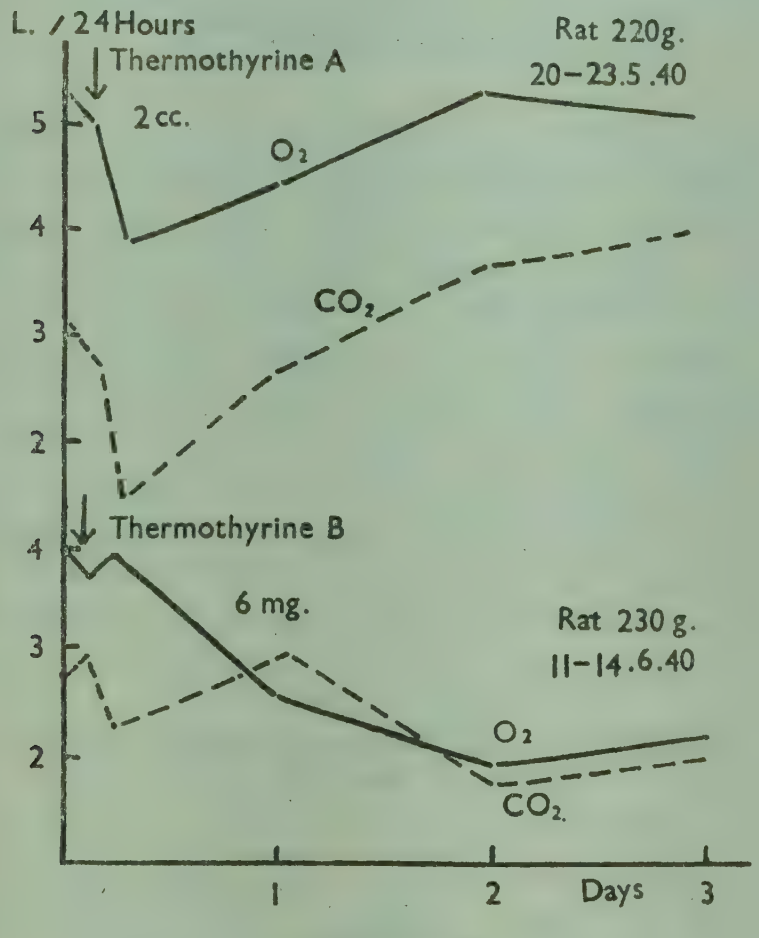


DIAGRAM 39.
Effect of thermothyrin A and B on the gas-exchange of rats

163. BELAK AND ILLENYI, A.: *Biochem. Z.* (1935), 281, 27.

CHAPTER XV

THE ROLE OF THE THYROID GLAND IN THE REGULATION OF THE BODY TEMPERATURE AGAINST OVER-HEATING

ELSEWHERE we reported on experiments which demonstrated that the blood serum of freezing as well as of over-heated animals contains active thyroid substances which, in the one, considerably augment, and in the other, deeply lower, the sugar consumption of isolated hearts of rabbits. (See Table XXXVI.) Whilst our experiments regarding the *efficacy of cold sera* were re-examined and found confirmation (which was partly histological and partly experimental), the fact that the *blood serum of warmed-up animals lowers* the sugar consumption of the heart, and that it is also conditioned by the thyroid gland, remained completely unnoticed.

The possibility of analyzing the question with more exact methods, and the observation that the thyroid gland produces

TABLE XLVI

Effect of 5–10 c.c. Blood Serum of warmed-up Animals on O_2-consumption of Muscle of Blood Receiver.

Experiment No.	Date 1938	Blood donor	Blood receiver	O_2-consumption mm.³ per gm. muscle per hour		
				Before serum injection	30 mins. after serum injection	Difference %
8	16 XI	Aw	71	266	218	—18
9	17 XI	Bw	72	238	194	—18
10	18 XI	Cw	73	219	199	— 9
			74	236	192	—18
11	21 XI	Dw	75	243	212	—12
12	23 XI	Ew	76	205	202	± 0
			77	255	198	—22
			78	243	189	—22
			79	271	215	—20
13	24 XI	Fw	80	188	201	+ 7
			81	232	225	— 3
14	26 XI	Gw	82	227	208	— 8
			83	278	204	—26

active substances which hinder combustion, caused us to renew our investigations regarding the hormonal factors in the regulation of the body temperature against over-heating.

The course of the experiments was the same as the one described on page 88, the only difference being that here the blood donors were exposed for 3–4 hours before the blood removal to an external temperature of 31–33°C. and that their body temperature rose by 1–2°C. Here as well a gastrocnemius of a

rabbit suspended in O_2 served as a test for the efficacy of the "warm sera"; its O_2-consumption was manometrically determined by Warburg's method.

The result of the experiments is shown in Table XLVI.

The experiments demonstrated that the serum of warmed-up animals possesses the faculty of hindering combustion in the muscle, because each analyzed serum led, in at least one of the blood receivers, to a reduction of combustion in the muscles.

This proves that the regulation of the body against over-heating also causes an active substance to appear in the blood which, when transferred to normal animals, diminishes combustion in the muscles; this hindrance of oxidation continues to exist also after separation of the muscle from the nervous system.

The above result confirms and explains that of Plaut and Wilbrand[153] who, after over-heating, found a fall of metabolism below the initial value (i.e., when the rectal temperature had reached normal again). Hence the authors concluded that there was a *chemical regulation against over-heating,* which they called the "second chemical heat regulation".

TABLE XLVII

Effect of 5–10 c.c. Blood Serum of warmed-up Thyroidectomized Animals on O_2-consumption of Muscle.

Experiment No.	Date 1938	Blood donor (thyroidless)	Blood receiver	O_2-consumption mm.³ per gm. muscle per hour		
				Before serum injection	30 mins. after serum injection	Difference %
15	30 XI	Hw	84	210	202	— 8
			85	210	186	—11
			86	227	202	—11
16	1 XII	Iw	87	218	238	+ 9
			88	238	224	— 5
			89	211	221	+ 5
17	2 XII	Kw	91	197	214	+ 8
			92	237	217	— 8
			93	229	223	— 2
18	5 XII	Lw	94	231	205	— 9
			95	249	242	— 2
19	7 XII	Mw	96	262	269	+ 2
			97	268	262	— 2

Bearing in mind the experiments reported above, one cannot doubt any longer that such a regulation is possible, nay, probable, and that it is a matter of humorally transferable chemical substances formed in the heat, which we described earlier[140] as a "cooling hormone".

This question arose: Does this active substance originate in the thyroid gland, as our former experiments made us presume? A repetition of the experiments just reported with the utilization

of thyroidectomized blood donors was bound to bring a decision to this question. In Table XLVII we see the result of these experiments, in which we removed the thyroid glands of the blood donors and injected normal blood receivers with the serum obtained after over-heating.

We see that the serum of five thyroidectomized, warmed-up blood donors proved to be ineffective on thirteen blood receivers. Not in a single case could a hindrance of combustion be observed which was worth while mentioning.

The active substance which hinders combustion at high external temperatures is derived therefore from the thyroid gland.

Our next task was to investigate the effect of these active warm sera on the whole animal. For this purpose we used as blood donors dogs kept for 30–45 minutes in a bath of 46° C. until their body temperature had risen by 3–4° C. The blood serum of these overheated animals was injected subcutaneously in quantities of 50–60 c.c. to dogs with a tracheal cannula substituted by a buffer cannula for the duration of the gas-exchange experiment. In these experiments on an unbound animal, lying quietly, the O_2-consumption alone was determined, by Krogh's method. In a second series of experiments on curarized dogs, O_2-consumption and CO_2-formation were recorded continuously by means of the metabolic apparatus,[164] under simultaneous measurement of the blood pressure.

TABLE XLVIII
Effect of Warm Sera on O_2-consumption of Dogs.

Date	O_2-consumption in c.c. per min. at 0°C., 760 mm. Hg		Difference %	Maximum effect after hours	Blood donor
	Before serum injection	After serum injection			
15 XII 1939	132	106	—19·6	5	Normal
9 I 1940	117	81	—30·7	4	
27 I 1940	118	82	—30·6	3	
8 III 1940	136	118	—13·0	4	
11 III 1940	141	104	—26·2	5	Without hypophysis
13 III 1940	166	148	—12·0	2	Without thyroid
18 III 1940	134	117	—11·0	3	

These experiments were meant to throw light on the question whether the thyroid gland alone is necessary for the formation of active serum substances or whether, as in the case of cold sera, the co-operation of the hypophysis is also required. Therefore, we used for these experiments partly normal blood donors and partly blood donors whose thyroid gland or hypophysis was removed.

Removal of the thyroid gland was performed, the parathyroids

being left intact; the hypophysis was removed according to the surgical technique described by Mosonyi.[165]

The results can be seen in Table XLVIII as well as Diagrams 40 and 41.

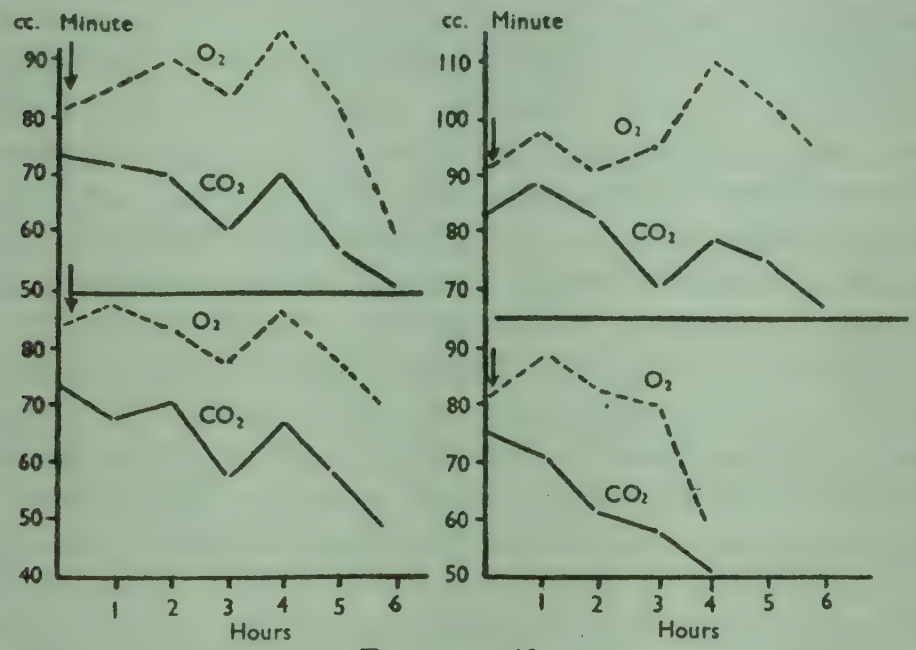


DIAGRAM 40.

Effect of warm sera on the gas-exchange of curarized dogs. 60 c.c. of warm sera intravenously at ↓

From these experiments it follows that the *warm sera of both normal and hypophysectomized dogs* bring about a considerable reduction of combustion, amounting to 20–30 per cent, which reaches its maximum in the third to fifth hour. It is highly remarkable, and it proved to be of importance later on, that the warm sera led, at least in the spring months, to a considerable fall of the respiratory quotient, because, particularly in the first few hours, the O_2-consumption is not diminished; only the CO_2-formation drops, presumably because in this season the O_2 saved is used up for the formation of glycogen.

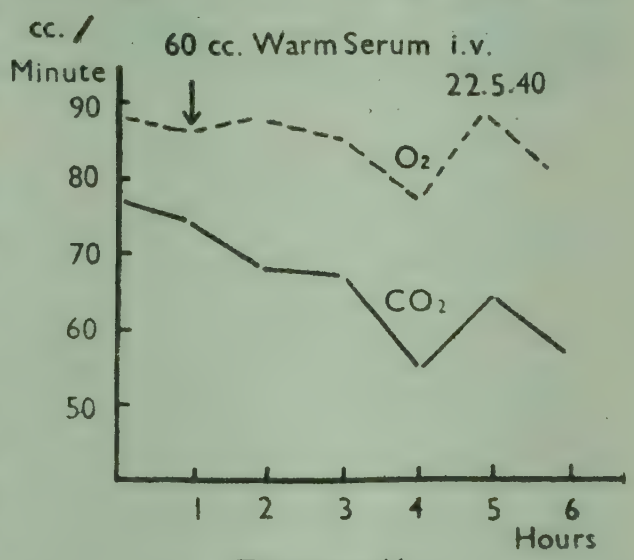


DIAGRAM 41.

Effect of warm serum obtained from hypophysectomized donor on a normal curarized dog.

The experiments made with the serum on thyroidectomized blood donors confirm the fact, shown earlier, that the *active substance of the warm sera comes from the thyroid gland*, whereas experiments on

hypophysectomized blood donors demonstrated (in contrast to the active substance of the cold sera) *that the activity of the hypophysis is not required for its formation.*

During the past three years further interesting data concerning thermothyrin A secretion have been published by B. Berde.[165a,165b] The physiological bearing of thermothyrin A secretion was demonstrated in heat tolerance experiments. In numerous preliminary overheating experiments pairs of guinea pigs were chosen with equal heat tolerance. One of these animals was thyroidectomized and the overheating experiments repeated. The heat tolerance of the thyroidectomized animal was reduced: its body temperature rose higher and for a longer period than that of the control. Furthermore the upper limit of the environmental temperature, which can be tolerated without elevation of the body temperature, was found to be lower for thyroidectomized guinea pigs and rabbits than for normal animals. The reduced heat tolerance could not be corrected by administration of thyroxin but could be restored by thermothyrin treatment. When the parasympathetic nerves of the thyroid gland are sectioned, no thermothyrin A secretion can be observed.[165c] Similarly the secretion of this hormone is inhibited by feeding methylthiouracil.[165d,165e]

164. Mansfeld, G.: *Klin. Woch.* (1933), **12**, 668.
165. Mosonyi, J.: *Pfluegers Archiv.* (1939), **242**, 92.
165a. Berde, B.: *Hungarica Acta Physiologica,* (1947), **1**, 52.
165b. Berde, B.: *Schweiz. Med. Wschr.* (1947), **77**, 1367.
165c. Berde, B.: *Experientia* (1948), in the press.
165d. Berde, B.: *Nature* (1947), **159**, 748.
165e. Berde, B.: *Experientia* (1947), **3**, 245.

CHAPTER XVI

THE SIMILARITY BETWEEN THE EFFECT OF THE THERMOTHYRINS AND THAT OF THE WARM SERA

Two different angles of approach had shown that the thyroid gland contains substances which are probably secreted into the blood, and which lower the oxidation processes in the organ cells, so we naturally thought that these substances might be identical. To examine this we analyzed the extracts obtained from the hydrolysates of the thyroid gland and the serum of over-heated animals and made a comparison with their efficacy on isolated organs.

All we saw was that the warm serum of rabbits, when injected into normal animals, brings about a fall of combustion in the muscle. The question arose whether thermothyrin was also capable of so doing.

These experiments, and those that follow in this chapter, were made at a time when a separation of the two substances of the thyroid extract had not yet been done, so that the thermothyrin used was a mixture of thermothyrins A and B.

We see from Table XLIX that thermothyrin diminishes the O_2-consumption of the muscle as early as 30 *minutes after its incorporation in exactly the same way as the warm serum.*

TABLE XLIX

Effect of Warm Serum and Thermothyrin on O_2-consumption of Isolated Organs.

Date 1940	O_2-consumption mm.³ per gm. muscle per hr.		Difference %	Date 1940	O_2-consumption mm.³ per gm. muscle per hr.		Difference %
	Before	30 mins. after			Before	30 mins. after	
	warm serum				thermothyrin		
12 VI	258	206	—20	21 V	258	209	—18
12 VI	249	212	—15	22 V	293	211	—21
26 VI	226	175	—22	22 V	238	186	—22

We showed (see Diagram 34) that thermothyrin leads to the destruction of the oxidation-increasing effect of thyroxin in the liver. We had, therefore, to investigate whether the warm serum produced the same effect. The identity of both effects is seen in Diagram 42.

These experiments indicated that it was highly probable that the active substances contained in the thyroid extracts and in the warm sera were identical. Whilst, however, thermothyrin came

from the hydrolysate of thyroid glands and was free from colloid substances, the warm sera contained all the serum proteins.

In order to be able to speak of an identity of the active substances, we had to investigate whether the warm sera retained their efficacy after removal of the proteins and lipoids.

For this purpose we freed the warm sera from their proteins using ethyl alcohol. After filtration of the precipitate, the alcohol was distilled off *in vacuo* until the volume was equal to that of the original serum. The solution was turbid, due to the lipoids; it was shaken with ether; after freeing the solution from the ether it became clear and free from biuret; we then used it for our experiments. We tested the effect of these lipoid-free and protein-free sera on the gas exchange of white rats in the metabolic apparatus of Belak and Illenyi.[163] The animals received a standard diet consisting of 8 gm. white bread, 5 gm. oats and 5 c.c. milk per 100 gm. body-weight, which secures a regular gas exchange. (R. Oberdisse.[97])

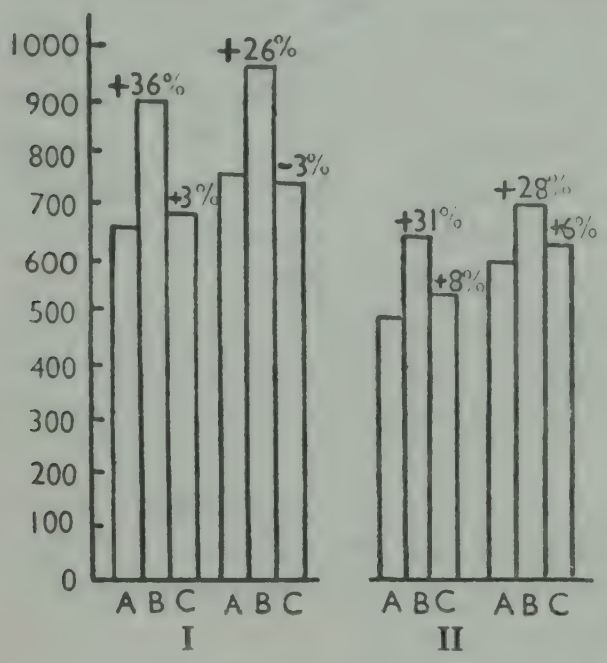


DIAGRAM 42.

Destruction of thyroxin effect in the livers of 4 rabbits.

I. By warm serum.
II. By thermothyrin.

A. Normal O_2-consumption of liver-cells.

B. 25 hours after 1 mg. thyroxin.

C. 1 hour after warm serum or thermothyrin.

When carrying out these experiments, by which the effectiveness of thermothyrin is easily determined, attention must be paid to the fact that the animals are kept at an outer temperature of 28–30°C. during the determination of metabolism. A glass vessel serves as container for the animals so that their movements can be observed. If it is sufficient to determine the production of CO_2, it is best according to Oberdisse[97] to conduct the air current by a by-pass as soon as the animal becomes restless and only to reconnect with the absorption vessels when the animal is calm again. We measure the time in which the quantity of produced CO_2 has been absorbed. If we want to determine both the O_2-consumption and the CO_2-production as we proceed, it is important to give the animal container a rocking motion, in the same way as small children are made drowsy by rocking the

cradle. It is to be noted that if the animal container should be submerged in a water bath of 30°C. and the rat is sitting on the glass or even only its long hairless tail (which constitutes an important organ of heat regulation among rats) is in contact with the glass, its chemical heat regulation is by no means eliminated. The rat will freeze as if it were not in an agreeable air temperature, but in a cool bath of 30°C. This has to be observed, for obviously only by elimination of chemical heat regulation can a reduction of combustion by thermothyrin be proved. Either there must be an air-jacket between the animal container and the water bath, or the glass vessel must be insulated inside by a bad thermal conductor, for instance a cork plate, but only so far that the animal can be observed well from above.

TABLE L

Effect of 2 c.c. Protein-free Warm Serum on the Metabolism of Rats.

Date	Maximum Difference %		Warm serum from
	O_2-consumption	CO_2-formation	
4 XI 1940	—40	—	Rabbit
8 XII 1940	—23	—20	Dog
12 I 1941	—50	—12	Dog
1 IV 1941	—43	—26	Dog
7 IV 1941	—20	—19	Dog
12 V 1941	—17	— 8	Rabbit

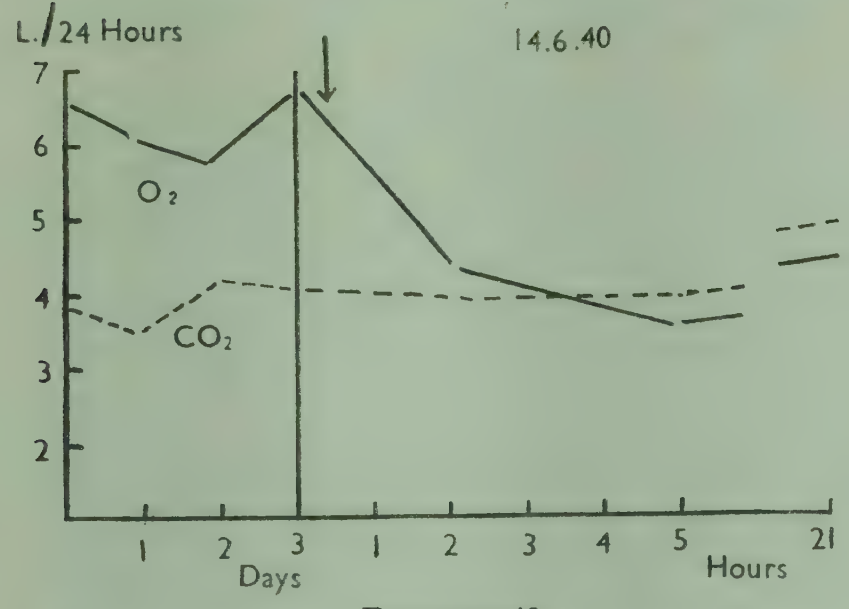


DIAGRAM 43.

Effect of protein-free warm serum of dog on the gas-exchange of a rat.

2 c.c. at ↓

A large number of experiments, about which we shall report fully later on and some of which are given in Table L, demonstrated that the efficacy of the sera is in no way affected by the removal of the colloid substances.

As can be seen from Diagram 43, the protein-free warm serum diminishes the O_2-consumption by the very considerable amount of 41 per cent with a latency of a few hours, exactly as the native sera did in the previously mentioned transfer experiments. (See Diagram 40.)

This fact was of importance in two respects. Firstly, it strengthened our assumption that the active substance of the serum is identical with the thermothyrin found in the thyroid gland; secondly, it then showed how to isolate oxidation-hindering substances from the warm serum in a pure form, and how to establish chemically their identity with the thermothyrins.

More will be said about it in the next chapter.

CHAPTER XVII

THE PREPARATION OF THERMOTHYRINS FROM THE THYROID GLAND AND FROM THE BLOOD SERA OF MAN AND ANIMAL

By ANNA MANSFELD, Ph.D. (Budapest)

THE experiments reported in the last chapter pointed strongly to the likelihood that the oxidation-hindering active substances found in the thyroid gland—the thermothyrins A and B—were identical with those which exist in the serum of warmed-up dogs and rabbits in the metabolic experiments. However, this could be considered as proved only if we succeeded in producing these substances in a pure form and in identifying them chemically as well. My first task was to prepare these compounds—known only from the point of view of their effect—from the thyroid gland, and to examine whether the same substances, which are delivered to the blood in the warm season or in warm surroundings, could be produced from the sera that were found effective and keep their hormonal nature.

(*a*) PREPARATION OF THE THERMOTHYRINS FROM THE THYROID GLAND

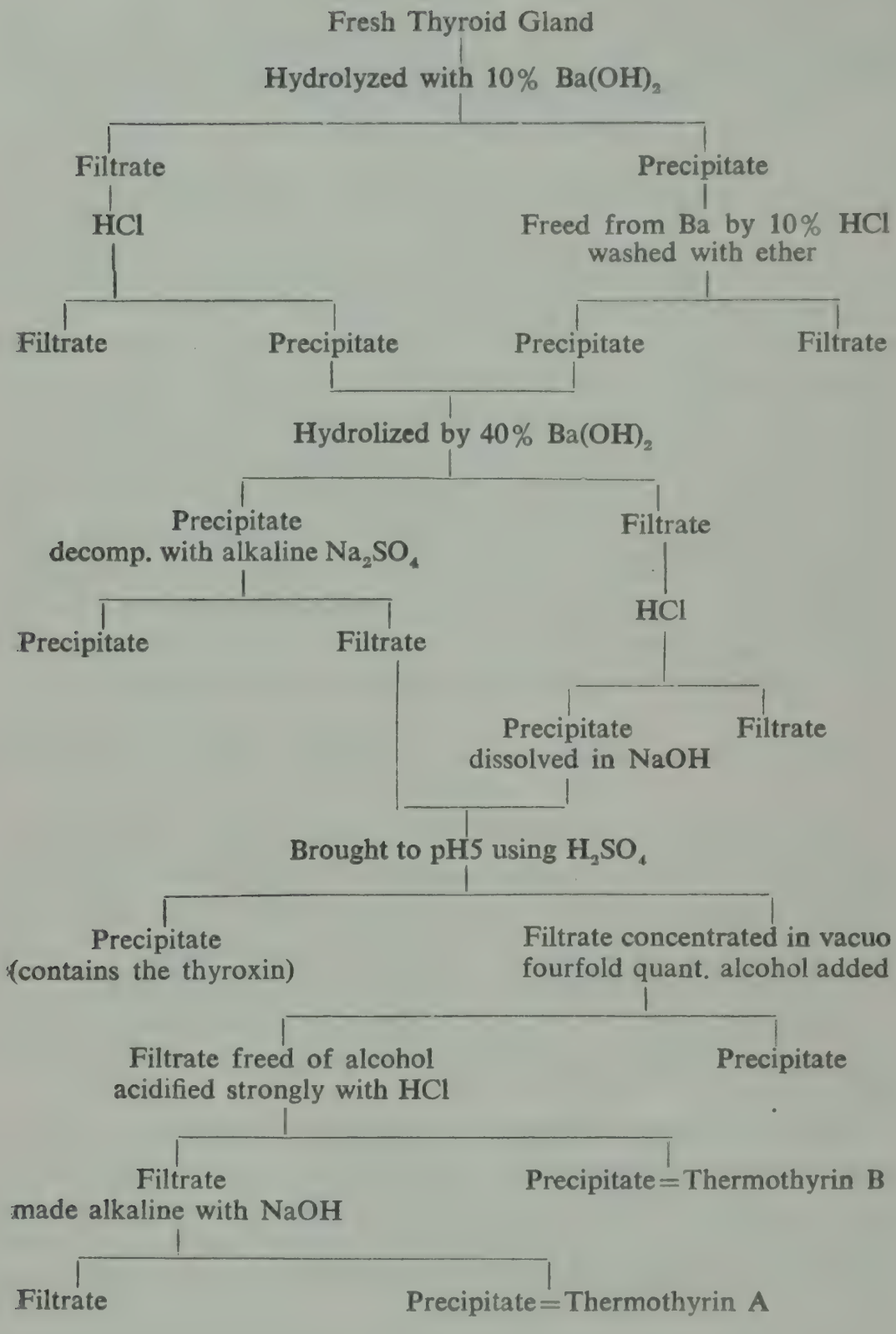
As raw material we used thyroid glands of oxen and pigs, raw, freed from the fat tissues and rendered as blood-free as possible through washing with tap water. Before using them, they were at first kept for 2–3 weeks in a cold store at a temperature of about +4°C., but later on they were preserved in methyl alcohol.

We proceeded according to Harington's method until the precipitation of the thyroxin occurred at pH 5, as shown in the scheme overleaf.

The thyroxin-free solution was distilled *in vacuo* to one-fifth of its volume and precipitated with 96 per cent alcohol until no more precipitate (Na_2SO_4) was formed. The filtrate was concentrated, using a vacuum pump, and the solution was made up to a volume which corresponded to 5 gm. fresh thyroid substance per 1 c.c.

By adding alkali to this solution we obtained a precipitate containing thermothyrin A, whereas by addition of acid another precipitate is formed which contains thermothyrin B.

The precipitates were thoroughly washed with water, dried, extracted with methyl alcohol, and filtered.


Fresh Thyroid Gland
Hydrolyzed with 10% Ba(OH)2
Filtrate
Precipitate
HCl
Freed from Ba by 10% HCl
washed with ether
Filtrate
Precipitate
Precipitate
Filtrate
Hydrolized by 40% Ba(OH)2
Precipitate
decomp. with alkaline Na2SO4
Filtrate
HCl
Precipitate
Filtrate
Precipitate
dissolved in NaOH
Filtrate
Brought to pH5 using H2SO4
Precipitate
(contains the thyroxin)
Filtrate concentrated in vacuo
fourfold quant. alcohol added
Filtrate freed of alcohol
acidified strongly with HCl
Precipitate
Filtrate
made alkaline with NaOH
Precipitate = Thermothyrin B
Filtrate
Precipitate = Thermothyrin A

When we concentrated this solution of the thermothyrin A carefully on a water bath, white crystals were precipitated on cooling. After 24 hours the crystals were separated from the methyl alcohol by centrifugation and dried.

A similar crystalline precipitate was obtained from the solution of thermothyrin B in methyl alcohol by adding a two-fold quantity of acetone.

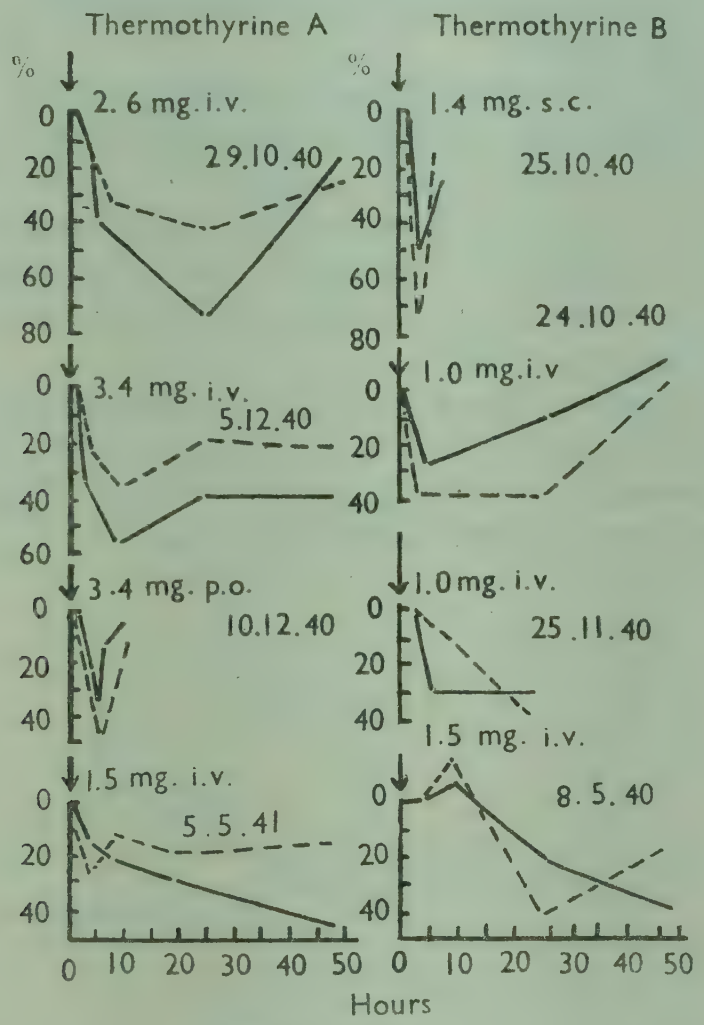


DIAGRAM 44.
Percentage alteration of the gas-exchange of rats after administration of thermothyrins.
—— O_2. ---- CO_2.

(*b*) ESTIMATION OF METABOLIC ACTIVITY

Both compounds in pure condition were hardly soluble in water, so that for their identification in the metabolic experiment they had to be introduced either intravenously in a fine emulsion or enterally with a probe, which proved to be almost as effective.

The experiments were made in the metabolic apparatus of Belak and Illenyi on white rats which received the usual standard diet.

We see the result of these experiments in Diagram 44.

We see that these active substances, prepared from the thyroid gland, in mg. doses brought about a reduction of combustion up to 75 per cent.

(c) Preparation of the Thermothyrins from the Blood Serum of Man and Animal

The next question was: Can we consider these substances, in fact, as hormones of the thyroid gland which get into the blood as its internal secretions?

Since former experiments demonstrated that the blood sera of animals which had been exposed to heat diminish combustion after removal of the protein and lipoid substances, it seemed likely that they contained these thyroid substances. My next task, therefore, was to find out whether it was possible to make preparations of thermothyrins from the protein-free sera which proved effective in the metabolic experiment.

The first results of these investigations showed that we could produce from the blood serum of *dogs, rabbits, and men* the same active substances, in crystals, the production of which from the thyroid glands has just been described.

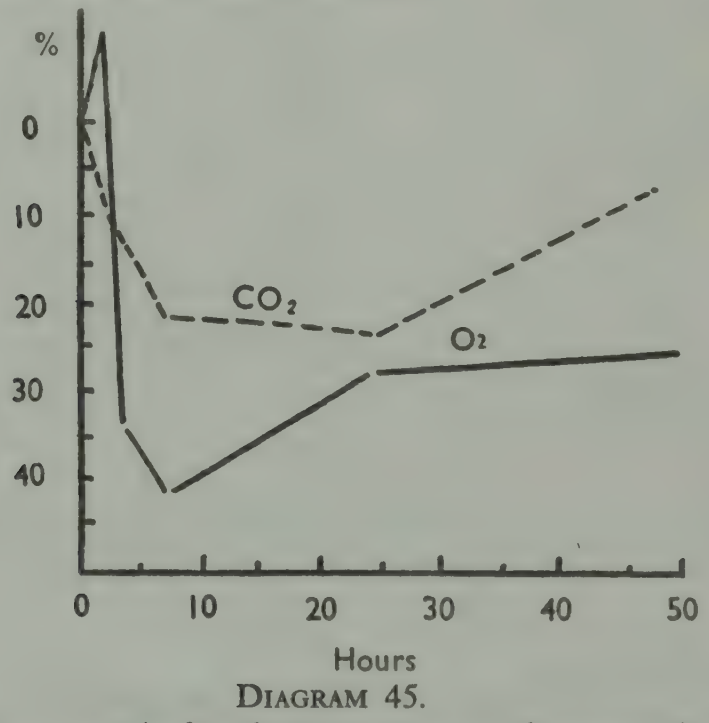


Diagram 45.

Effect of 2 c.c. protein-free human serum on the gas-exchange of rat. (Serum of Dr. Sós obtained on 14th May, 1941.)

Immediately after a hot bath of 40–45° C. a corresponding quantity of blood was taken from dogs and men. Rabbits were kept for 2–3 hours in a heat box before the blood removal.

The removed blood (20–25 c.c. human blood was sufficient) was allowed to coagulate; the serum obtained was precipitated with the four-fold quantity of 96 per cent alcohol, filtered, freed

from the alcohol *in vacuo*, and made up to its original volume. The solution was turbid, due to the alcohol-soluble lipoid substances; it was washed with ether in a separating funnel, and freed from the residual ether at room temperature. In Diagram 45 the metabolic effect of human warm serum on a rat is shown.

Through addition of both acid and alkali to the protein-free sera, which are metabolically effective, precipitates were obtained which were soluble in methyl alcohol. The alkaline precipitate—similar to thermothyrin A obtained from the thyroid gland—crystallized out during the concentration of the methyl alcohol, whereas the acid precipitate—similar to thermothyrin B—crystallized out only after addition of acetone.

The compounds obtained from the sera proved to be identical with the active substances of the thyroid gland not only by their metabolic effect but also by their chemical behaviour.

In Table LI we show the effectiveness of the thermothyrins prepared from the blood serum of dogs on the gas exchange of white rats.

TABLE LI

Effect of Thermothyrins prepared from Blood Serum.

Dose mg.	Maximum Difference %	
	O_2-consumption	CO_2-formation
Thermothyrin A		
1·4 i.v.	—13	—22
3·4 per os	—46	—52
3·0 i.v.	—41	—33
3·6 „	—42	—23
0·4 „	—12	—18
1·8 „	—19	
Thermothyrin B		
3·0 i.v.	—38	—30
0·8 „	—44	—38
1·5 „	—46	—43
0·2 „	—45	

(*d*) IDENTIFICATION OF THE ACTIVE SUBSTANCES OF THE SERUM AS HORMONES OF THE THYROID GLAND

Do the active substances of the serum come from the thyroid gland?

To solve this question we used in our experiments the blood sera of both *normal* and *thyroidectomized, warmed-up animals.* The warmed sera obtained were freed from proteins, washed with ether, and after establishing their efficacy in the metabolic experiment, we prepared the thermothyrins with the minimum loss possible in the way earlier described, and weighed them.

The result is shown in Table LII.

We see that the sera of thyroidectomized animals were without effect in the metabolic experiment and that it was impossible to

obtain thermothyrins from them. From the warm serum of normal rabbits and of men, however, thermothyrins could be prepared, and the sera proved to be effective in every case.

These experiments leave no room for any doubt that the oxidation-hindering substances of the blood sera come from the thyroid gland and are identical with those which we prepared from the thyroid gland.

TABLE LII *

The effect of 2 c.c. Warm Serum on the Metabolism of the Rat and the amounts of Thermothyrin obtained from the Serum.

Date 1941	Maximum Difference %		Amount obtained from 100cc. serum		Remarks
	O_2-consumption	CO_2-formation	Thermothyrin A, mg.	Thermothyrin B, mg.	
			Normal		
20 III	—30	—34	22	9	Rabbit
12 V	—30		9·3	0·7	Rabbit
14 V	—41	—26	1·5	6	Man
16 V	—25	—20	4·6	1·5	Man
			Thyroidectomized		
12 III	+12	+ 3	0	0	Rabbit
17 III	— 6	— 6	0	0	Rabbit
30 IV	+13	+14	0	0	Rabbit
3 V	— 9	— 9	0	0	Rabbit

* With regard to this Table and Table LIII, it must be noted that the quantities of thermothyrins obtained cannot claim to be exact, because, on the one hand, their preparation was not a quantitative one, and, on the other, the quantities obtained must be divided by 10 in order to get the correct value of the active substances. A further analysis showed that the crystalline precipitates still contained 80-90 per cent of ash. These experiments demonstrated, however, the important fact that the metabolic efficacy of the sera is linked up with the presence of these active substances and that the active substances obtained from the serum are identical with the substances obtained from the thyroid gland through hydrolysis, not only from the point of view of their metabolic effect, but also in respect of their solubility.

Another question remained: What role do the two thermothyrins play in the heat regulation?

As former experiments demonstrated, there are two different conditions under which the thyroid gland produces active substances which hinder oxidation. The first is warm surroundings as is shown by the effect of the warm sera. The second is the coming of spring, with surroundings at normal temperature, as was proved both in the novocaine experiment and in the metabolic experiment. This was fully discussed in Chapter XI. (See Table XLIV.)

In the experiments that followed we had to investigate which of the two thermothyrins would present the active substance of the

warm sera, i.e., the " cooling hormone ", and which corresponded to the " summer hormone " of the thyroid gland, i.e. the one conditioned by the season.

For this purpose we took the blood sera in different seasons and in different temperatures of surroundings, tested them as to their effectiveness and determined the quantities of thermothyrins that could be prepared from them.

The result of these experiments is to be seen in Table LIII.

TABLE LIII

Effect of Blood Serum (2 c.c.) on Metabolism and the Content of Thermothyrin at different Seasons.

Date	Temperature of surroundings	Maximum Difference %		Amount obtained from 100 cc. serum	
		O_2-consumption	CO_2-formation	Thermothyrin A, mg.	Thermothyrin B, mg.
		Winter			
2 XII 1940	Cool	+ 6	+ 9	0	0
20 II 1941	Cool	—10	— 3	0	0
28 XI 1940	Warm	—40		10	0
11 XII 1940	Warm	—26	—20	20	0
12 I 1941	Warm	—50	—12	11	0
		Spring			
7 III 1941	Cool	—24	—36	0	4
3 V 1941	Cool	—57	—67	0	7·5
14 V 1941	Cool (Man)	—41	—26	1·5	6
12 V 1941	Warm	—30		9·3	0·7
20 III 1941	Warm	—30	—34	22	9
16 V 1941	Warm (Man)	—25	—20	4·6	1·5

The experiments show the following results. The sera obtained in cool surrounding temperature (under 20° C.) were ineffective *in winter* and contained neither thermothyrin A nor thermothyrin B. In the spring they strongly hindered oxidation and contained only thermothyrin B. Sera obtained at high temperature (35–45° C.) were effective in the winter and contained thermothyrin A, but not thermothyrin B. Warm sera obtained in the spring contained both thermothyrins.

It followed that thermothyrin A entered into the blood independently of the season if the animal was exposed to high external temperature. It presented the " cooling hormone " of the thyroid gland.

Thermothyrin B, on the contrary, was seen to be secreted by the thyroid gland in the warm season, independent of high external temperature and therefore had to be regarded as the " summer hormone " of the thyroid gland.

We report now about investigations, the purpose of which was a fuller acquaintance with the chemical qualities of the thermothyrins.

(*e*) Purification and Chemical Properties of the Thermothyrins *

An anlysis of the thermothyrins prepared from thyroid glands and from blood sera showed that despite their high degree of efficacy, as shown in Tables LI–LIII, they still contained 80–90 per cent ash.

A further purification was difficult because the quantities of the active substances prepared by hydrolysis from the thyroid glands of cattle were rather small and because the thyroid glands available for preparations in the winter months contained no thermothyrins at all, which coincided with the results of physiological experiments. This forced us to collect in the warm spring and summer months as many thyroid glands as possible so as to have sufficient raw material for the winter months. This procedure was a turning point in my work. It brought far better possibilities, due to which in the end a pure preparation of both active substances could be achieved.

When I worked on the thyroid glands collected during the summer and preserved in methanol, the result deteriorated from month to month. It seemed out of the question that the active substances, which endured a hydrolysis of 18 hours' duration without decomposition, would suffer damage within a few months from being preserved in methanol at room temperature. So we had to assume that these active substances were incorporated with the methanol during their long preservation. My assumption was strengthened when at one time I caused the decanted methyl alcohol to evaporate and injected 0.1 gm. of the residue into a white rat. It brought about such a powerful fall of combustion that the animal could no longer be saved even in the heat-box. Thereafter I used to extract the thyroid glands with boiling methyl alcohol and not by hydrolysis. In this way more substantial quantities of the active substances could be obtained. Shortly afterwards we succeeded in preparing them in a pure crystalline state. As methanol dissolves not only the thermothyrins but also many inorganic substances, we had to find a solvent for their purification, in which the active substances dissolved but the inorganic ones did not. Acetone proved to be suitable for this purpose.

We proceeded in the following way: 2 kg. freshly chopped thyroid substance were freed from blood, as far as possible, and boiled for 10 hours with 3 litres methanol. When it had cooled off it was filtered and evaporated to dryness *in vacuo*. The residue, dissolved in about 300 c.c. boiling acetone, was filtered hot and

* Cf. Dr. Anna Mansfeld: *Schweiz. Med. Wschr.* (1946), 76, 439, and *Nature* (1946), No. 3994.

concentrated to about 40 c.c.; then it was filtered hot again and left alone for 24 hours at room temperature. A white precipitate formed. It was filtered through a Hirsch's filter and crystallized out from 20 c.c. boiling acetone.

In this way beautiful white shining crystals were obtained, which proved to be identical in their effect with the thermothyrin B prepared from thyroid glands by hydrolysis and also from acetone after manifold crystallizations.

The mother-liquor remaining after the filtration through Hirsch's filter was concentrated further to 20 c.c.; the thermothyrin B which might have precipitated at room temperature was filtered off, and the solution was preserved in the refrigerator for 24 hours. The precipitate was dissolved in cold acetone; it was evaporated to half volume and crystallized again in the refrigerator. In this way I obtained thermothyrin A.

The crystals obtained in this way proved to be free from ash and displayed an efficacy in the metabolic experiment which, the longer it went on, the more it exceeded the efficacy of the impure crystals that were obtained previously. The fall of metabolism caused by the impure thermothyrins amounted to 30–40 per cent and ended on the third day, whereas the same quantity of pure crystals kept combustion down by 50 per cent up to the fifth day and did not lose their effect completely until the ninth day.

Before I report on the chemical properties of the thermothyrins, I should like to draw attention to the fact that both these active substances are not found in the thyroid gland as complex compounds (though this may be so in the case of thyroxin), but are pre-formed compounds soluble in methyl alcohol.

The result of the determination of the melting-point was for thermothyrin A, 53° C.; for thermothyrin B, 66° C. These melting-points remained unaltered after repeated crystallizations.

An elementary analysis which I made at the Chemical Institute of the University of Pecs under the leadership of Prof. L. Cholnoky, by Pregl's method, gave the following results:

For Thermothyrin B

4.403 mg. substance gave 13.755 mg. CO_2 and 5.785 mg. H_2O, i.e., C = 85.22%, H = 14.70%

2.874 mg. substance gave 8.922 mg. CO_2 and 3.805 mg. H_2O, i.e., C = 85.38%, H = 14.81%

2.745 mg. substance gave 8.530 mg. CO_2 and 3.622 mg. H_2O, i.e., C = 85.30%, H = 14.70%

2.988 mg. substance gave 9.300 mg. CO_2 and 4.025 mg. H_2O, i.e., C = 84.93%, H = 15.07%

3.183 mg. substance gave 9.920 mg. CO_2 and 4.232 mg. H_2O,
i.e., C = 85.05%, H = 14.88%

2.861 mg. substance gave 8.915 mg. CO_2 and 3.833 mg. H_2O,
i.e., C = 85.03%, H = 14.99%

Mean values obtained: C = 85.15%
H = 14.87%

Total 100.02%

The compound consisted entirely of C and H. Its empirical formula is $C_{20}H_{42}$, for which the corresponding theoretical values are C = 85.01%, and H = 14.98%.

Whether the molecule of the compound contains a multiple of the formula was investigated by determination of the lowering of freezing point in cyclopentadecanone.

1.788 mg. substance in 31.426 mg. cyclopentadecanone gave 4.1°C. decrease.

M = 296.

2.326 mg. substance in 47.794 mg. cyclopentadecanone gave 4.0°C. decrease.

M = 259.

Average value obtained: 277.

The molecular weight calculated for $C_{20}H_{42}$ is 288. The molecular formula of thermothyrin B therefore is $C_{20}H_{42}$ and not a multiple thereof.

For Thermothyrin A

2.576 mg. substance gave 7.622 mg. CO_2 and 3.200 mg. H_2O,
i.e., C = 80.74%, H = 13.90%

3.045 mg. substance gave 9.000 mg. CO_2 and 3.824 mg. H_2O,
i.e., C = 80.65%, H = 14.05%

2.058 mg. substance gave 7.662 mg. CO_2 and 3.210 mg. H_2O,
i.e., C = 80.79%, H = 13.88%

Mean value obtained: C = 80.72%
H = 13.94%

Total 94.66%

This compound contains C and H and one atom of oxygen, and its empirical formula is either $C_{20}H_{42}O$ with the corresponding theoretical values C = 80.46% and H = 14.18%, or the formula $C_{20}H_{40}O$ with the theoretical values C = 81% and H = 13.60%.

Since this slight difference cannot be decided by combustion experiments this question will have to remain open until the constitution has been clarified.

The lowering of freezing point in cyclopentadecanone was: 1.690 mg. substance in 30.255 mg. cyclopentadecanone gave 4.1 °C. decrease.

$$M = 290.$$

The molecular weight calculated for the formula $C_{20}H_{40}O$ is 296. Thus the molecular weight found for the thermothyrin A corresponds closely to the calculated value.

A further clarification of the constitution of these highly active endocrine substances is in progress.

CHAPTER XVIII

THE EFFECT OF THERMOTHYRINS ON ISOLATED ORGAN CELLS

THE reported experiments seem to confirm that the thermothyrins play a role in heat regulation, partly through their faculty of reducing the basal metabolism, and partly through their thyroxin-antagonistic effect. The latter did not seem to be of importance for physiology alone. There was the probability that the thermothyrins, due to their small production, played a part in the pathology of Graves' disease. There was a temptation to try to influence the disease therapeutically through the thermothyrins. But this is offset by a fact which is well known to clinical workers: that the condition of a patient suffering from Graves' disease becomes worse precisely in those circumstances which, according to our experiments on man and animal, lead to an increased production of the new hormones, i.e., high external temperature and arrival of the warm season.

This indicated that things were not as simple as they looked on first sight, and that further investigation of the effect of the thermothyrins was required firstly on cell oxidations. This seemed the more necessary as some therapeutic experiments made in the meantime with the new hormones on these patients were a failure.

All our experiments to date demonstrated that when thermothyrins are incorporated into the whole organism, they bring about a decrease of oxidation. We had, however, still to investigate how the cell oxidation was altered by administration of the new hormones to living isolated organ cells in Warburg's experiment.

These investigations brought a big surprise. As can be seen in Table LIV both hormones in all the concentrations tested led *to a considerable increase of the O_2-consumption.*

In these experiments we used organ sections after Warburg, and Ringer's solution as suspension fluid; the hormones were given in concentrations of 10^{-6}–10^{-17}. We used for these experiments partly the crystalline compounds described in the preceding chapter, partly solutions obtained from the thyroid gland, the hormone content of which was determined in the metabolic experiment on a rat with sufficient accuracy for the purpose.

Thus the same active substances which bring about a considerable reduction of combustion when applied to the whole animal lead on isolated cells to a powerful increase of the O_2-consumption.

Our next task was to find the causes of this contrary effect.

TABLE LIV

Effect of Thermothyrin on O_2-consumption of Sliced Tissue.

Thermothyrin	Concentration gm. per cc.	Qo_2 without thermothyrin	Qo_2 with thermothyrin	Difference %	Organ
A	10^{-11}	2·6	3·9	+33	Liver
A	10^{-12}	5·8	6·9	+21	Liver
B	10^{-9}	5·8	7·7	+32	Kidney
B	10^{-9}	6·8	9·2	+35	Liver
B	10^{-13}		8·9	+31	Liver
B	10^{-14}	5·35	6·7	+26	Liver
B	10^{-15}	4·4	6·7	+51	Liver
B	10^{-17}		6·6	+48	Liver
B	10^{-9}	5·8	7·8	+34	Kidney
B	10^{-9}	6·2	7·5	+21	Brain

CHAPTER XIX

A FURTHER NEWLY DISCOVERED ROLE OF THE THYROID GLAND

FROM the experiments reported in the preceding chapter we had to draw the conclusion that the whole organism, or at least the co-operation of several organs, is necessary in order to bring about the combustion-antagonistic effect of the thermothyrins. It did not seem unlikely that the preliminary condition for the metabolic effect of the thermothyrins observed on the whole organism was the function of an endocrine gland. We therefore investigated at first their metabolic effect on *thyroidectomized animals.*

The experiments brought us a great deal nearer the solution of the enigma. It was demonstrated that the thermothyrins, which as a rule lower combustion in normal animals far below normal (see Diagram 44), *bring about an increase of oxidation in thyroidectomized animals, as we have seen in isolated organ cells.*

This inverse effect of the thermothyrins on thyroidectomized animals occurred in all our experiments without exception, if at least two months had elapsed after the removal of the thyroid

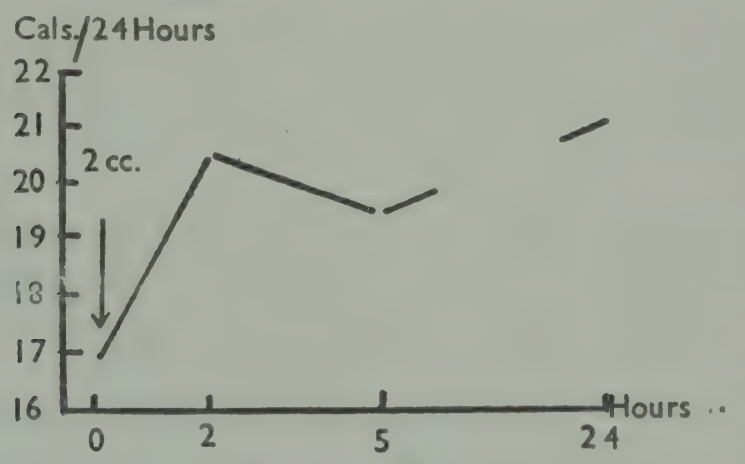


DIAGRAM 46.
Thyroidectomized rat.
At ↓ 2 c.c. thermothyrin A solution.

gland. A shorter period is not sufficient for achieving an inverse effect; when this happens, however, the increase of combustion due to the thermothyrins is as constant as the decrease in a normal animal. The long latent period required for the inversion of the metabolic effect was a good indicator for a further clarification of this interesting phenomenon.

In Diagrams 46 and 47 we show some examples of the metabolism-increasing effect of the thermothyrins on thyroidectomized rats.

These experiments were of importance from two points of view. Firstly, because we recognized in the thermothyrins active substances of the thyroid gland, which, in absence or insufficiency of thyroid activity, lead to an increase of metabolism, which until now we saw only as a result of the effect of thyroxin. This seemed of pathological importance, particularly in the case of Graves' disease with its increased metabolism. We shall come back to this question later.

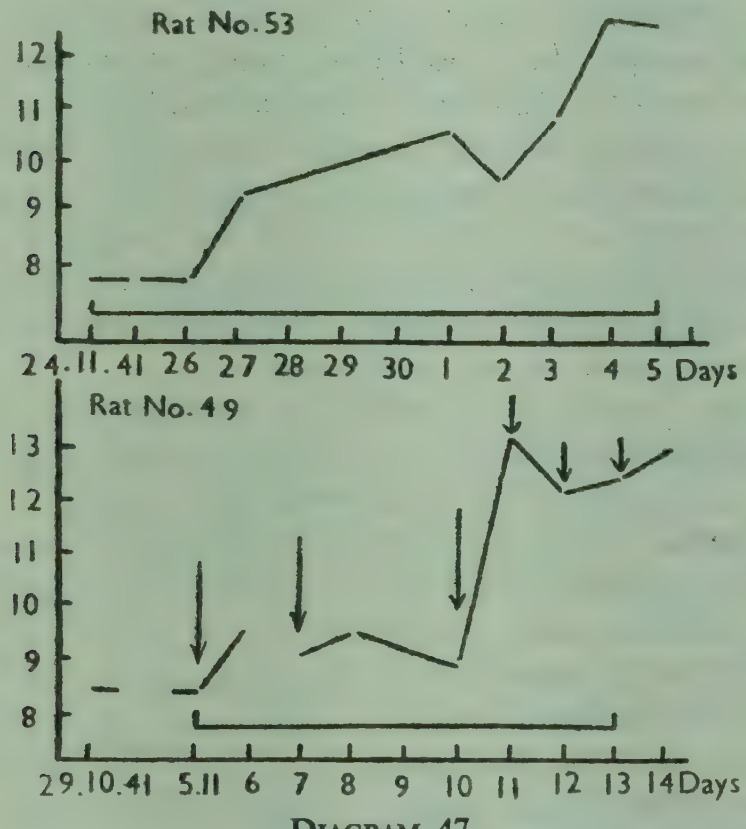


DIAGRAM 47

Thyroidectomized rats. Ordinate: cal. per 100 gm. per 24 hours.
Above: 2 c.c. thermothyrin A daily (Date, 24/11/41–5/12/41).
Below: 2 c.c. thermothyrin B at ↓ (Date 29/10/41–14/11/41.)

Further, these experiments demonstrated *a new role of the thyroid gland by which two of its own active substances acquire the faculty of intervening actively in the mechanism of chemical heat regulation.*

Our next goal was to find out the conditions of this newly recognized activity of the thyroid gland.

CHAPTER XX

ANALYSIS OF THE NEWLY RECOGNIZED FUNCTION OF THE THYROID GLAND

FROM our experiments mentioned in Chapter X regarding hormonal regulation against cold, it followed that the hypophysis also possesses an active substance which increases combustion. So we searched first to see whether the thermothyrins displayed *their oxidation-increasing* effect through the mediation of the hypophysis in cases where the thyroid gland was missing. For this purpose we analyzed the effect of the thermothyrins on rats from which the thyroid gland and hypophysis had been removed.

We found that the thermothyrins increased the O_2-consumption in a thyroidectomized animal without the co-operation of the hypophysis, which coincides with the fact that the thermothyrins —as we have just seen—increase combustion in isolated organ cells. An example is shown in Diagram 48.

The next question was: Can the function of the missing thyroid gland be substituted by one of its active substances, or does it depend on the *activity* of the thyroid gland itself?

This question is of importance on principle, because it may well be imagined that certain active substances of the thyroid gland might be available in the organ cells whereby the thermothyrins lower the combustion, but it may also be that the incorporated thermothyrins are transformed into combustion-antagonistic hormones only in the thyroid gland itself.

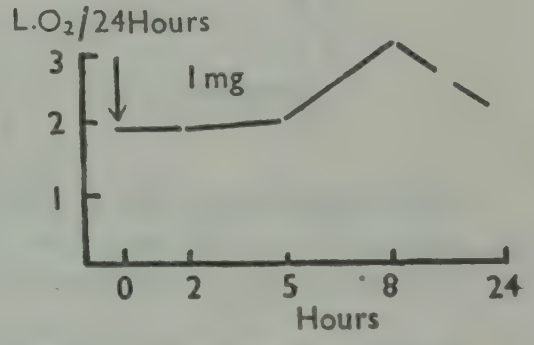


DIAGRAM 48.
Effect of 1 mg. thermothyrin B on O_2-consumption of a hypophysectomized and thyroidectomized rat.

The assumption that the thyroid gland delivers substances which are necessary for the physiological effect of the thermothyrins is supported by our experiments in which *we tried to substitute* the missing thyroid gland in rats through *implantation of rats' thyroid.*

It was demonstrated that, shortly after the implantation of the thyroid gland into the abdominal wall, the effect of the thermothyrins became normal again, even if the implantate was

fully absorbed due to bad vascularization, so that 2–3 weeks after the implantation nothing was left except the silk thread with which we fastened it to the lower surface of the abdominal musculature. This pointed to the possibility that we were dealing here with substances which became absorbed from the implant. This became still more probable when we found that small thyroid fragments of dogs could also replace the thyroid gland of rats.

In Diagram 49 we see a return of the effect of the thermothyrins to normal after implantation of rat thyroid.

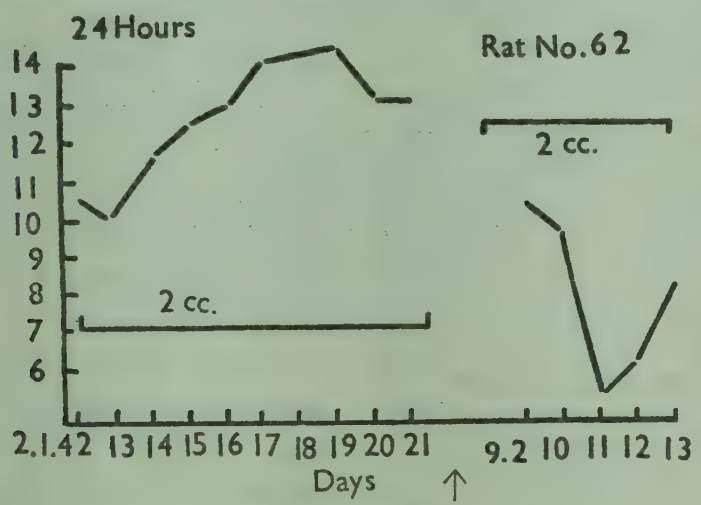


DIAGRAM 49.
Thyroidectomized rat receiving 2 c.c. thermothyrin daily.
(Date 2/1/42–13/2/42).
At ↑ implantation of thyroid on 31/1/42.
Ordinate: Cals./100 g./24 hours.

In Diagram 50 we see, on the same animal, the gradual development of the inverse effect of the thermothyrins upon thyroidectomy and its return to normal after implantation of a small piece of dog thyroid.

The next question was: Is thyroxin necessary for bringing about the physiological effect of the thermothyrins?

In order to decide this, we increased the gas exchange in thyroidectomized animals by using thermothyrins and then administered thyroxin in order to see whether the metabolic effect would return to normal.

These experiments gave a highly important result. It showed that both thermothyrins, whose metabolic effect until now proved to be identical both on normal and thyroidectomized animals, suddenly behave basically differently in a thyroidectomized animal after administration of thyroxin. Whereas thermothyrin B, after administration of thyroxin, leads to a fall of combustion in a thyroidectomized animal in the same way as in a normal one, the combustion-increasing effect of thermothyrin A remains unaltered and is even strengthened through thyroxin.

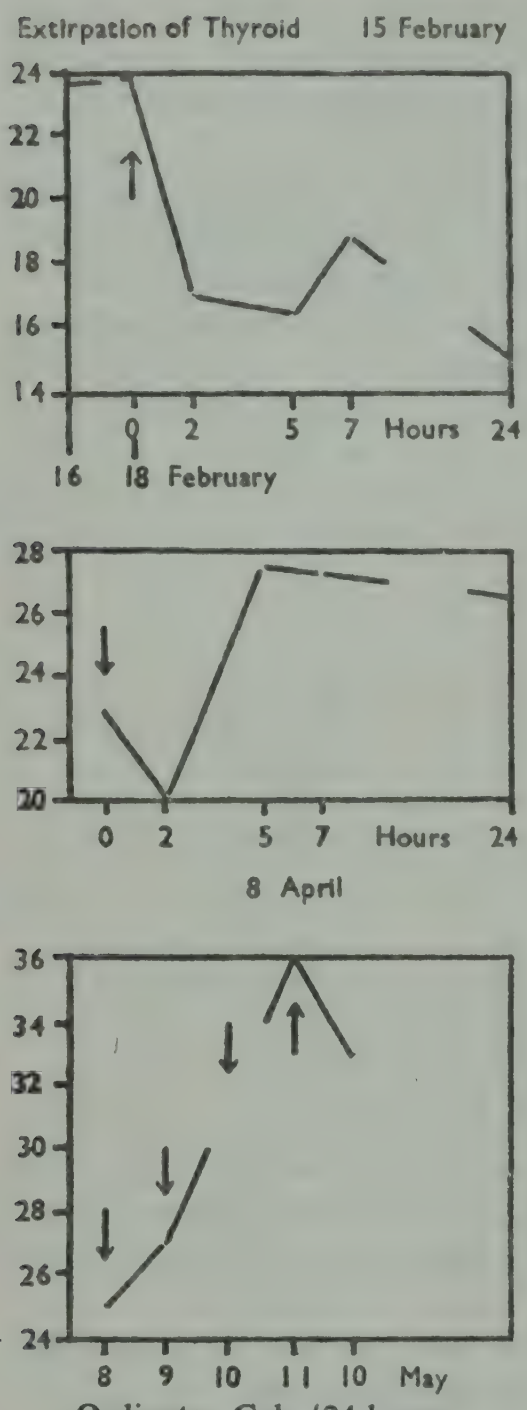


DIAGRAM 50.

2 c.c. thermothyrin B at ↓ ↑

Diagram 51 shows the effect of thermothyrin B on a thyroidectomized rat before and after administration of thyroxin.

Administration of iodothyreoglobulin acted in the same way as thyroxin, the former having been prepared according to the prescription of Oswald and kindly put at our disposal by Prof. Went (Debrecen).

In Diagram 53 we see the peculiar fact that administration of thyroxin led *to a reduction of combustion* if given at a time when the effect of thermothyrin B was still manifest.

Thus thyroxin creates the conditions which enable the thermothyrin B to lower combustion corresponding to its physiological role.

On the other hand, thyroxin proved to be completely ineffective with regard to thermothyrin A which, as we see from Diagram 54, increases combustion in a thyroidectomized animal despite administration of thyroxin.

Diagram 55 shows the effect of iodothyreoglobulin. The gas exchange, increased through thermothyrin A, suffered a decisive reduction as a result of iodothyreoglobulin; it was, however, not in a position to hinder an increase of combustion after a renewed intake of thermothyrin A. The dosages were perhaps insufficient.

We have seen that the absorption of all active substances of the implantate *signifies a complete substitute of the*

missing thyroid gland, also in respect of the thermothyrin A. The question arose, however, which are the substances of importance for it?

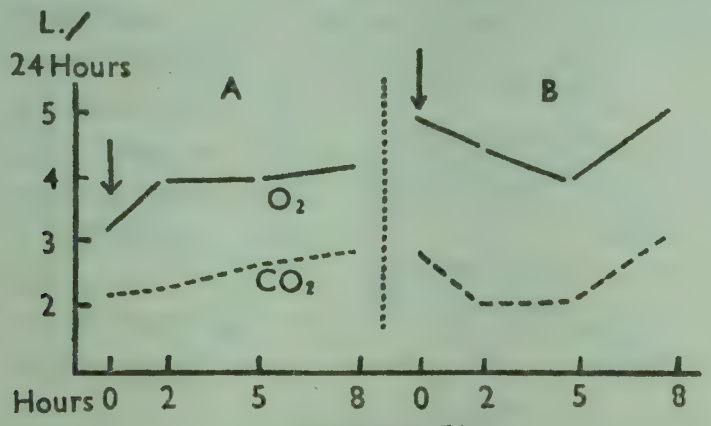


DIAGRAM 51.

Effect of 1 mg. thermothyrin B at ↓ on the gas-exchange of a thyroidectomized rat.

A. Before thyroxin treatment.

B. After thyroxin treatment (2 × 0.3 mg.)

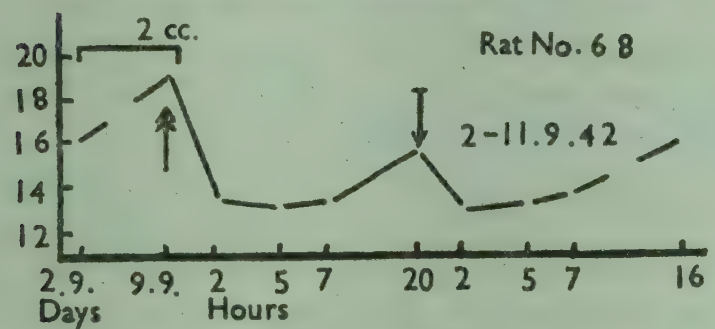


DIAGRAM 52.

Thyroidectomized rat treated with 2 c.c. thermothyrin B daily for 7 days.

↑ 10 mg. iodothyreoglobin. ↓ 2 c.c. thermothyrin B.

Ordinate: Cals./24 hours.

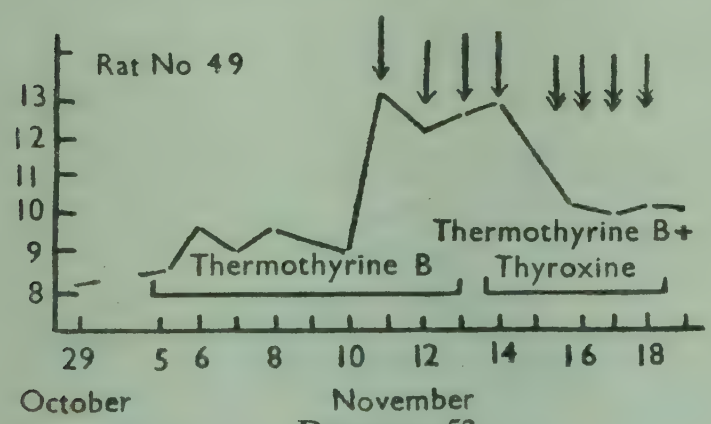


DIAGRAM 53.

Thyroidectomized rat.

↓ { 2 c.c. thermothyrin B on 5th, 7th, 10th, 11th, 12th, and 13th November. Thyroxin on 14th November.

↓↓ Thermothyrin B + thyroxin.

Ordinate: Cals./100 g./24 hours.

After thyroidectomy it takes months before the effect of the thermothyrins suffers an inversion. This would point to its being a matter of secretion of the thyroid gland, which remains active in the organism for a long time, even after its supply has been interrupted. In the first place we thought of the colloid of the *thyroid gland* which, since the discovery of thyroxin by Kendall, has been pushed into the background as an inner secretion of the thyroid gland. Trendelenburg remarks that "many of the facts of the physiology of the thyroid prove that it can travel in the opposite direction as well, i.e., from the follicular space via the epithelial cell into the body fluids". (*Die Hormone*, Vol. II, p. 8.)

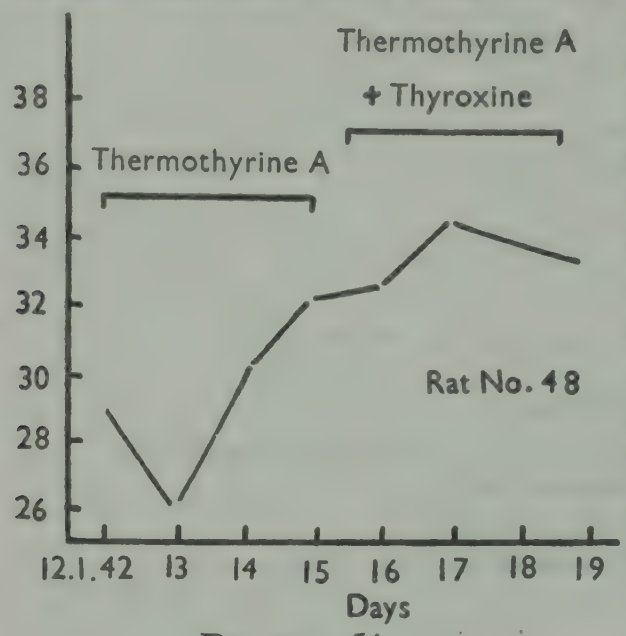


DIAGRAM 54.
Thyroidectomized rat.
2 c.c. thermothyrin A daily without and with thyroxin. Date 12/1/42–13/1/42.
Ordinate: Cals./24 hours.

It was mainly the investigations of Biondi,[166] Langendorff,[167] and Huerthle[168] which pointed to the fact that the colloid as a proper inner secretion of the thyroid gland passes into the lymph spaces from the follicles either between the principal cells or

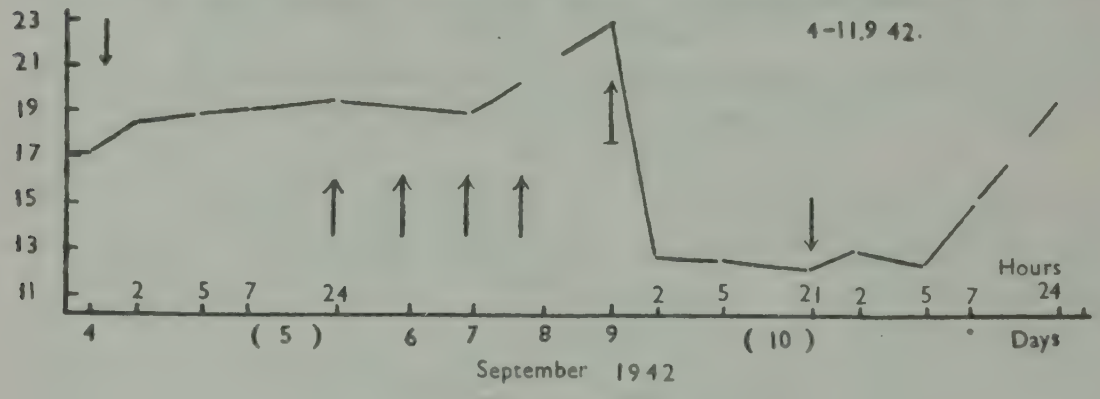


DIAGRAM 55.
Thyroidectomized rat.
↑ 2 c.c. thermothyrin A. ⇑ 10 mg. iodothyreoglobulin.
Ordinate: Cals./24 hours.

through liquation of the epithelium and rupture of the follicle (Huerthle). Later on the fundamentally important and praiseworthy investigations of Grab[169] furnished the experimental biological proof that the colloid is, in fact, an inner secretion of the thyroid gland and serves as a storing place and transport vehicle for the iodine-containing active substances of the thyroid,

primarily for thyroxin; laden with these active substances, it gets into the blood circulation in order to deliver there the active substance proper.

These investigations caused us to examine whether the colloid obtained from thyroids can substitute the newly recognized function of the thyroid gland.

For this purpose we prepared colloid from the thyroid glands of oxen following the procedure of Tatum.[170] I should like to describe this procedure in detail.

The thyroid glands of oxen, freshly slaughtered in the abattoir, were brought to the Institute in Ringer's solution containing 0.3 per cent tricresol. After washing off the blood they were freed from the fat tissue and cut piece by piece into sections of about 20μ in thickness on an ice-cold microtome. These were put in ice-cold Ringer's solution, prepared in large flat dishes. Ringer's solution, with the slices swimming on the surface, was kept in motion by gentle shaking. By staining samples we convinced ourselves that all the colloid fell from the follicles or dissolved within 10–15 minutes. Most of the slices were then fished out, and in order to enrich the (Ringer's) solution as much as possible, we used it for further slips until the surface was again fully covered with them. This was repeated five or six times. Finally, the solutions and the slices were again united and then separated through the centrifuge. The slightly rose-coloured, slightly opalescent, solution was filtered through cotton wool, mixed with 0.3 per cent tricresol, and filled into phials of 5 c.c. Our hope that the solution would become sterile through addition of tricresol did not materialize, so we had to undertake a tyndallization. We held the phials for 1 hour in a water bath at 60°C. (±1°C.) and kept them afterwards for 24 hours at 37° C; then we held them again for half an hour at 60° C. and for 24 hours at 37° C. After this procedure they proved sterile and the solution retained its full efficacy, as we shall see.

The preparation corresponded to 0.1 gm. fresh thyroid per c.c. Its dry *organic* residue amounted to 1.3 per cent, its iodine content to 5.7 mg. per cent. The dissolved organic substance therefore contained 0.44 per cent iodine.

We injected 2 c.c. of this preparation into *normal* rats daily and tested its effect on combustion. *It proved completely without effect.* This does not in any way signify that it contained no thyroxin at all, but that the incorporated quantity of the colloid was very small (26 mg. dry organic substance with roughly 0.11 mg. iodine). According to Grab's calculation only one-third of the iodine in the colloid was derived from thyroxin; thus the animals received daily only 0.05 mg. thyroxin which, in our

experience, is equivalent to about one-sixth of the dose of thyroxin which has any effect on metabolism.

It is important to stress this, because despite this small quantity of iodine or thyroxin, *this intake substitutes completely the missing function of the thyroid gland.*

If we administer this colloid solution to *thyroidectomized* rats, on which we proved earlier the inverse metabolic effect of the thermothyrins, the animals behave again as normal ones, *so that their basal metabolism is considerably lowered by both thermothyrin A and thermothyrin B.*

We show this interesting effect of colloid in doses which in themselves have no effect on metabolism in Diagrams 56 and 57.

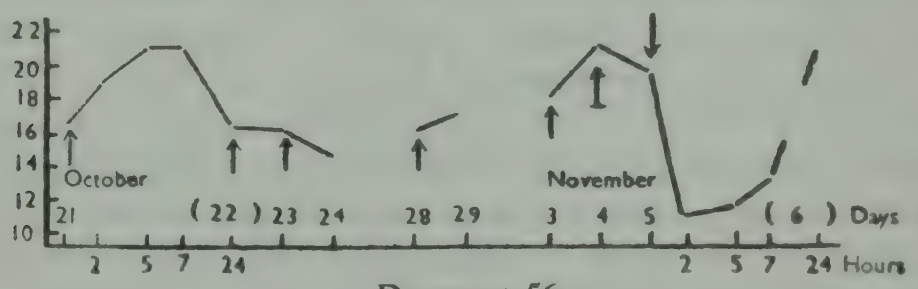


DIAGRAM 56.

Thyroidectomized rat.

↑ 2 c.c. thermothyrin B. ⇑ 2 c.c. thyroid colloid.

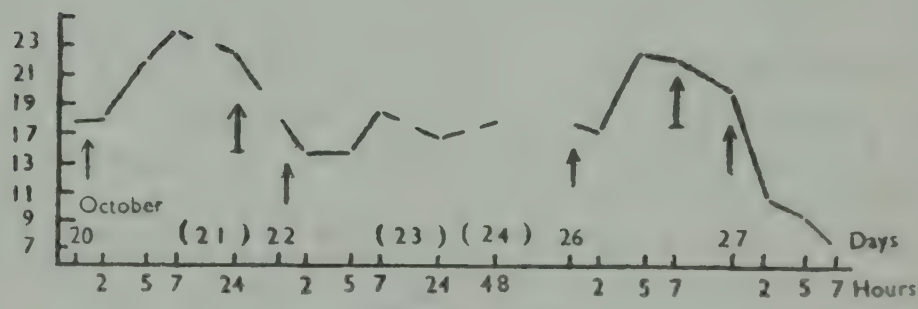


DIAGRAM 57.

Thyroidectomized rat.

↑ 2 c.c. thermothyrin A. ⇑ 2 c.c. thyroid colloid.

In Diagram 56 we see first the effect of an intake of thermothyrin B in a preliminary experiment on October 21, 1942. The maximum effect occurred 7 hours after the injection (increase of production of calories from 16 to 21) and was over after 24 hours. On October 22, 23, and 28 the animal received each time 2 c.c. solution of thermothyrin B with the same result (not shown to save space). Due to these repeated injections the production of calories rose and on November 4, 1942, 24 hours after the last thermothyrin injection, it reached almost 22 calories per 24 hours. After determining the gas exchange on that day, the animal received 2 c.c. thyroid colloid which was without effect on the gas

exchange. An injection of thermothyrin B on the following day led to a fall in the production of calories up to nearly 10 calories. The effect of the thermothyrin after preliminary treatment with the colloid presents an inversion of the effect on an animal without such preliminary treatment, and is also over after 24 hours.

We see in Diagram 57 the same inversion of the effect of the thermothyrin A under the influence of the colloid of the thyroid gland.

A further proof the effectiveness of colloid could be produced. We refer to Table LIV, from which it follows that addition of thermothyrins to surviving slices of organs brings about an increase of combustion. When we administered thyroid colloid to slices the thermothyrins were no longer capable of producing an increase of the O_2-consumption but remained either without effect or reduced combustion, as can be seen from Table LV.

TABLE LV

Effect of Thermothyrins on the O_2-consumption of Sliced Liver suspended in a Solution of Thyroid Colloid.

Thermothyrin	Concentration gm. per cc.	Qo_2 without thermothyrin	Qo_2 with thermothyrin	Difference %
A	10^{-3}	4·13	3·07	—25
A	10^{-4}	4·08	3·25	—20
A	10^{-5}	6·83	6·89	0
A	10^{-6}	8·24	9·04	+ 9
B	10^{-3}	4·08	3·43	—18
B	10^{-4}	6·83	7·38	+ 8
B	10^{-5}	4·13	3·60	—12

On the one hand our experiments demonstrated that the inner secretion of the thyroid gland is necessary for the whole organism in order that the thermothyrins should diminish combustion in their physiological role in the heat regulation; on the other, we see in isolated cells that the presence of thyroid colloid in the cells is a preliminary condition for this physiological effect of the thermothyrins.

W. Grab[169] concluded from his investigations that the colloid is only a vehicle for the transportation of active crystalloid substances of the thyroid gland. Without doubting that thyroxin, and perhaps also other active substances containing iodine, are bound to the colloid when they leave the gland, I believe that the colloid itself must be regarded as an active secretory product of the thyroid gland, which displays the described effect in the cells. The main thing for us was that we saw that neither thyroxin nor iodothyreoglobulin are equivalent to the colloid, because these only partly substitute the missing thyroid, whilst the colloid proved to be a complete substitute of the thyroid gland for the efficacy

of both thermothyrins. That this active substance, superior to thyroxin, is of colloidal nature is stressed by the fact that its efficacy ends only two months after thyroidectomy. Such stubborn adhesion of a substance to the cell points to its colloidal nature and to the fact that as an assimilated part of the protoplasm it creates the conditions for the physiological effect of the thermothyrins. Should this assumption prove to be incorrect and should it be found that it is a crystalline substance, as the hormones generally are, then it must be bound very strongly indeed to the colloid. However, if it were to split as easily as thyroxin does, according to Grab, the long duration of its effect would not be understandable. We shall, of course, try to obtain through hydrolytic splitting of the colloid a crystalline substance which is capable of fully substituting the missing function of the thyroid gland. For the time being, however, *we must regard the thyroid colloid itself as the active substance of the thyroid gland which is absolutely necessary in order that the thermothyrins lower combustion and thus intervene actively in the process of heat regulation.*

166. Biondi: *Berlin. klin. Woch.* (1888), p. 954.
167. Langendorff: *Arch. Physiol.* (1889), Suppl., p. 218.
168. Huerthle, K.: *Pfluegers Archiv.* (1894), **56**, 1.
169. Grab, W.: *Arch. exp. Path. Pharm.* (1933), **172**, 586.
170. Tatum: *J. Biol. Chem.* (1920), **42**, 47.

CHAPTER XXI

THE THERMOTHYRINS AND GRAVES' DISEASE

We have now to examine how the new facts influence our views on the nature of Graves' disease. We do not intend to increase the number of theories which try to clarify this mysterious disease. We wish to ease the way to the co-operation of experimental and clinical medicine by a comparative and a critical survey of all available facts.

Two cardinal symptoms of Graves' disease, the struma and the increased metabolism, seemed to justify the assumption that it was a matter of over-production of thyroxin, the more so as thyroxin was known to be the only hormone to increase combustion in the organism at rest. This assumption was strongly supported by the therapeutic successes which were observed after surgical thyroidectomy or X-ray treatment of the thyroid gland.

The main reason, however, for scrutinizing this assumption was the fact that it was demonstrated that besides thyroxin there were two other active substances of the thyroid gland capable of raising combustion if, as we saw, the organism suffers from lack of thyroid colloid. This fact received our special attention because the Graves' thyroid gland is known to be poor in colloid.

Our further experiments on thyroidectomized rats demonstrated that a constant intake of thermothyrins, even in very small quantities (0.01–0.02 mg. daily), led to an increase of combustion up to and over 50 per cent, and parallel with it losses of weight of 30–35 per cent occurred within three weeks. Together with this severe condition of cachexia there was to be recorded a very strong irritability of the animals, so that even experienced staff were constantly wounded by bites.

It seemed to us of decisive importance to examine whether the thermothyrins were capable of simulating not only the effect on metabolism but also other symptoms of Graves' disease. We therefore investigated their effect on the pulse rate, because it was known that in animal experiments only very few authors succeeded in bringing about tachycardia by thyroxin.

For our experiments we used rabbits and determined by means of an electro-cardiograph the number of pulsations before and after the injection of the thermothyrins both on normal rabbits and on rabbits *whose thyroids had been removed at least two months earlier.*

The animals sat in a very narrow wooden cage which afforded no freedom of movement. The needle electrodes were stuck in the right shoulder and in the left hind leg. Before the actual day of the experiment the number of pulsations was determined for three days running; in the course of the day, without removing the

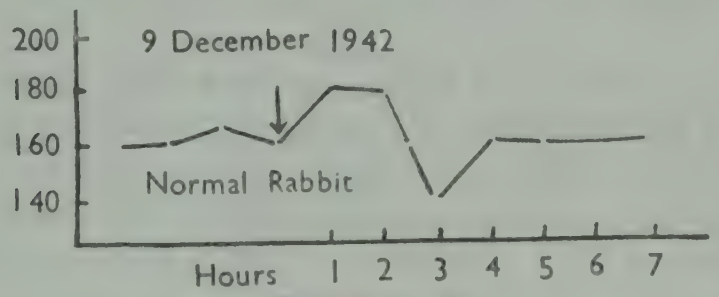


DIAGRAM 58.
10 c.c. thermothyrin A and B at ↓
Ordinate: Pulse rate.

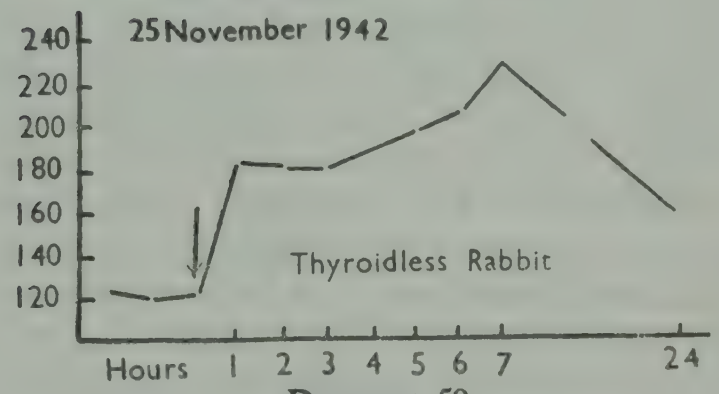


DIAGRAM 59
10 c.c. thermothyrin A and B at ↓
Ordinate: Pulse rate.

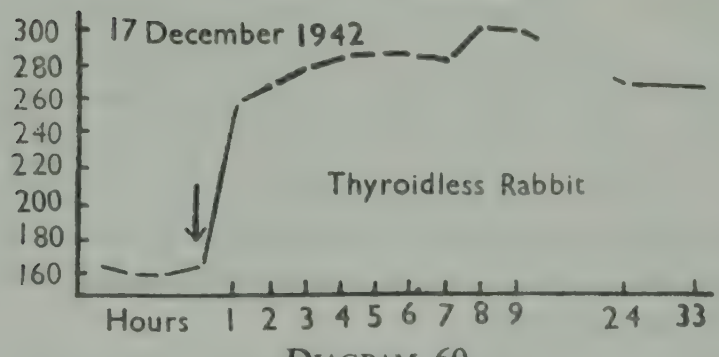


DIAGRAM 60
10 c.c. thermothyrin A and B at ↓
Ordinate: Pulse rate.

needles, 4–5 electro-cardiographic records were taken. Early in the morning of the day on which the experiment was done, a record was taken, the thermothyrins were injected, and the recording repeated every hour. The solution used contained both thermothyrins, about 0.5 mg. in 10 c.c. We see the effect on the number of pulsations of normal rabbits in Diagram 58, and of two thyroidectomized animals in Diagrams 59 and 60.

We see that it is possible to imitate by administration of

thermothyrins not only the increase of metabolism and the loss of weight of a patient; in cases where the function of the thyroid gland is missing, the thermothyrins bring about also a powerful increase of the pulse.

These experiments cause us to inquire whether the thermothyrins are responsible, at least partly, for the complex of the Graves' symptoms, and whether the lack of colloid is due simply to its insufficient formation rather than to an increased secretion.

Before we deal with a more detailed analysis of this possibility we have to ask ourselves whether such an assumption is at all justified, or even necessary; in other words, are we justified in entertaining a doubt that Graves' disease with its symptoms is brought about solely as a result of an over-production of thyroxin?

We shall analyze this query later on.

(*a*) Can an Increased Production of Thyroxin in Graves' Disease be Proved ?

The justification of this question lies in the fact that, as we have seen, thyroxin is not the only product of the thyroid gland which increases combustion. Therefore we must examine the literature and see whether anyone has succeeded in proving with sufficient certainty an increased production of thyroxin in the case of this disease.

If these thyroid glands form more thyroxin than normal ones, then one ought to be able to prove it either in the content of the gland or blood in Graves' disease.

As far as the proof of thyroxin in the blood is concerned, it is known that the blood of such patients contains about double the quantity of iodine than normal blood—20–40 γ per cent compared with 10–20 γ per cent of the norm (Veil and Sturm[171]). According to Lunde[172] and colleague, as well as Holst,[173] the organically-bound iodine is also augmented. This implied that the thyroid gland produces more thyroxin.

This conclusion would be relevant only if there were proof of a higher content of thyroxin in the blood of such a patient. Neither with R. Hunt's[174] acetonitrile reaction nor with the method of tadpoles was there certain proof. The positive findings made with the acetonitrile reaction were greatly reduced by the following facts: (1) that feeding of blood from *thyroidectomized rats* leads, as P. Trendelenburg[175] found, to an increased acetontrile reaction in the same way as a preliminary treatment with thyroid substance; (2) that not only the blood of patients suffering from Graves' disease but also from uraemia, asthma bronchialis, or diabetes, was antagonistic to acetonitrile.*

* Trendelenburg-Krayer: *Die Hormone*. II. p. 53. Springer, Berlin, 1933.

Whilst Read Hunt's[174] acetonitrile reaction gives useful results for the evaluation of preparations from the thyroid gland, it is unsuitable for the purpose of a specific proof of thyroxin in the blood.

The tadpole method, however, gives most useful results with regard to the proof of thyroxin, 1 part of thyroxin to 1 milliard parts water being sufficient to accelerate the metamorphosis. However, neither Rogoff and Goldblatt[176], nor Sharpey-Schaefer[177] could prove thyroxin in the blood with this method. This is rather remarkable because, as Grab[178] was able to demonstrate, it is possible to prove the presence of the augmented thyroxin both with R. Hunt's reaction and the metamorphosis test in the blood of dogs which were treated with the thyreotropic hormone.

After a critical survey of the available material and facts, Trendelenburg concludes (*Die Hormone*, p. 54) with the words: " A biological proof of an increased content of thyroid hormone in the blood in Graves' disease has not yet been furnished decisively."

The literature does not in any way affirm that an increased content of thyroxin in the blood of these patients has been demonstrated.

We are in no way better-off as regards proof of a higher thyroxin content in the thyroid. Whilst the assumption of a higher production of thyroxin in cases of Graves' disease, based on blood analysis, could at least be referred to the fact that the iodine content of the blood is increased, this support is non-existent in respect of the thyroid gland. We know, from the investigations of numerous authors, that the hyperplastic Graves' strumae, which are poor in colloids, contain absolutely and relatively very little iodine; according to Merke,[179], Holst,[173] and Lunde[180] they contain about ten times less iodine than normal thyroid glands.

In this connection we should like to point out that the thyroid glands of cretins, too, are very poor in iodine. (Baranovicky,[181] Wydler,[182] Jansen and Robert,[183] etc.). It is, however, conceivable that in Graves' disease the small quantity of iodine which is contained in the gland consists perhaps in its entirety of thyroxin, but this assumption is not supported by experience. The experiments on tadpoles must be considered as the most conclusive ones. In a large number of these experiments, as Trendelenburg rightly remarks, no attention was paid to the iodine content of the thyroid glands. It therefore could not be established whether the thyroid iodine in Graves' disease is in a compound which is more than normally active.

The experimental results of Marie Krogh and Lindberg[184] are highly remarkable. They established that Graves' thyroid glands

had *a smaller effect* on the O_2-consumption and the loss of weight in guinea pigs than an equal quantity of iodine in the form of normal thyroids of pigs. Thus the increase of the O_2-consumption brought about by a pig's thyroid gland was 1.3 per cent per mg. iodine but only 0.8 per cent in Graves' disease, whereas the biological effectiveness of the diseased thyroid gland rose to 1.5 per cent after a successful treatment with iodine.

Trendelenburg writes: "There is no proof whatsoever of a unique position of the thyroid gland in Graves' disease in that its effectiveness does not go beyond its content of iodine-proteins." It must be added that the experiments of Krogh and Lindberg[184] indicate, on the contrary, that hyperthyroid goitres contain less thyroxin than normal ones, and it is only after a successful *treatment with iodine that the production of thyroxin is restored and an improvement of the condition brought about.*[184a,184b]

I want to mention here that the operative successes in cases of Graves' disease cannot be attributed merely to a diminution of thyroxin-producing tissue, i.e., through a reduction of the thyroxin production itself. On the contrary, it is known from the investigations of Wagner,[185] Halsted,[186] as well as L. Loeb[187] that, after partial resection of the thyroid gland, the remainder shows symptoms of compensatory hyperplasia. Our own[188] experiments demonstrated that the resection of one half of the thyroid gland brings about an increase of iodine content in the remaining half, of 139 per cent on the average, which through administration of small iodine quantities could be raised to 225 per cent. The success of partial thyroidectomy in Graves' disease appears here in a different light because both therapeutic treatments, the administration of small quantities of iodine and the resection of thyroid tissue, would display their favourable effects by restoring the production of thyroxin.

In this connection it seems interesting to point out that a high altitude climate, with its relative O_2-deficiency, has, as is known, a very favourable effect on cases of Graves' disease. As fully discussed in Chapter I, it is known from many concurring experimental results that this O_2-deficiency leads to an increased activity of the thyroid gland. But there are also more direct proofs that in hyperthyreosis there exists a diminished, rather than an increased, "iodine function" of the thyroid gland. I refer to the investigations of Hamilton and Soley[189] who, after incorporation of radioactive iodine in man, determined the iodine storage in the thyroid gland. It was demonstrated that whilst the thyroid glands of normal human beings absorb the administered iodine and can keep it for many weeks, the thyroid gland of hyperthyreotics

has lost the faculty of storing iodine, so that their iodine content is increased only for a few hours. The thyroid gland of cretins behaves in the same way, so that the low iodine content in this case and in Graves' disease, to which attention has been drawn earlier, is explained by the inability to store up iodine.

The histological finding, that in Graves' disease we can see signs of an increased activity of the thyroid gland, was considered a strong support for the over-production of thyroxin. But we have no proof that this increased activity of the thyroid gland signifies at the same time an over-production of thyroxin, since we know that other hormones as well are produced by the thyroid gland. As already mentioned, it is quite possible that in Graves' disease only the colloid function of the thyroid gland is affected, whereas the thermothyrins are undisturbed and perhaps produced in increased quantities. A fact must be mentioned here which points to this possibility.

In Chapter XVII it was reported that we had succeeded in preparing thermothyrins from the blood sera of normal men and animals. We saw that in the *winter months* there is no thermothyrin B in the blood serum of either animal or man. On the other hand, during the months of December 1941 and January–February 1942, we found thermothyrin B in the blood serum of ten patients with Graves' disease, in each case in the same quantity as is found in normal persons only during the months March–November.

A critical analysis of the facts shows that an over-production of thyroxin in Graves' disease is in no way proved. Now that new facts have been made known, according to which the increased metabolism and the accelerated activity of the heart can be explained by a deficient activity of the thyroid gland, the question arises whether any symptoms of Graves' disease point to a diminished production of thyroxin or to a deficient function of the thyroid gland.

(*b*) Analogies Between Graves' Disease and Thyroxin Deficiency

We are now going to discuss a few facts that refute rather than favour the conclusion of an over-production of thyroxin.

First of all there is the fact that Graves' disease frequently appears after treatment with thyroid. This points to the existence of an abnormal fat appendage as a forerunner of this disease, which in itself speaks more for a diminished production of thyroxin than for an increase. Still less clear, in the light of an over-production of thyroxin, appear those cases of Graves' disease with fully

developed symptoms in which there is obesity. Recently A. Lublin[190] described, under the title *Hypo- and hyperthyroid obesity*, cases of " Graves' obesity " for which no explanation can be offered by the thyroxin theory of this disease. Furthermore, it is known that the warm seasons and warm surroundings deteriorate the condition of such a patient. If it were a matter of thyroxin intoxication, it would be difficult to understand why *conditions which lead, as we saw, combustion-hindering and thyroxin-antagonistic substances to penetrate the blood,* should lead to a worsening of Graves' disease. This clinical experience, to some degree, is analogous to those experiments in which, as was shown, the supply of hormones formed in the warm season or in warm external temperatures leads to an increase of combustion when the thyroid gland is missing.

In addition to obesity, there are other forms of diseases with deficient activity of the thyroid gland which show a relationship to Graves' disease. It is not rare, for instance, for it to develop into myxoedema. Baldwin[191] reported four such cases.

In the first part of this book the relationship between the activity of the thyroid gland and the formation of blood was discussed in detail, and we saw that deficient activity of the thyroid gland played an important part in pernicious anaemia.

Anaemia, which is a known attendant symptom of myxoedema, can also belong to the picture of Graves' disease. Indeed anaemia and cachexia are so conspicuous in this disease that von Basedow[192] himself originally considered this disease as a diathesis closely connected with chlorosis.

Highly noteworthy are cases in which hyperthyreosis associates with pernicious anaemia. Recently Stenstam[193] reported eight such cases and mentioned three cases with a combination of true Graves' disease with pernicious anaemia.

All this shows that a revision of our views on the pathogenesis of Graves' disease is indicated. As we now know, cardinal symptoms of this disease, such as, in the first place, the increase of metabolism, can be brought about not only by thyroxin but also by the thermothyrins when there is lack of thyroid colloid. We have to ask ourselves, therefore, whether Graves' disease can still be considered with justification as a result of an increased production of thyroxin or, perhaps, with Moebius, " the intoxication of the body of a person ill with Graves' disease is caused by a dysfunction of the thyroid gland due to the supply of a qualitatively different secretion.

This almost coincides with the view held by many recent authors that in Graves' disease a qualitative change in the activity of the thyroid gland, i.e., dysthyreoidism, has to be accepted.

Coincident with our findings is the assumption of McCarison[194] of a plurality of hormones of the thyroid gland. In Graves' disease these are discharged in abnormal quantitative proportions. This is also supported by the histological findings of Williamson and Pearce.[195]

The value of all this can only be decided in the clinic. Experimental medicine accumulates in vain facts which give rise to justified doubts regarding the prevailing theory of Graves' disease. Our experiments in which we succeeded in bringing about in an animal a condition similar to Graves' disease with the new hormones can only point the way; but we are in no position to produce proof that in men, too, a deficient function of the thyroid gland is in the forefront of the disease due to which the inverse effect on metabolism is brought about by the thermothyrins. If only it could be demonstrated that a parenteral supply of thyroid colloid or perhaps the implantation of healthy thyroid tissue eliminates the essential symptoms of the disease—as it does in animal experiments where the pathological effect of the thermothyrins on the metabolism is transformed back into normal— it would be justifiable to consider a deficient production of colloids as the primary disturbance in Graves' disease.*

I hope that the exposition of these new experiments and the fusion of their results with what is already known, as well as the discussion of many of the problems that obscure the complete understanding of Graves' disease, will help to stimulate and further a closer co-operation between experimental and clinical medicine in this field, which is one of the objects of this book.

* This experiment was carried out in the surgical department of the St. Rochus Hospital in Budapest in the autumn of 1943. The patient, a man of 53, was such a severe case of Graves' disease that it was not possible to make a resection of the thyroid gland. Metabolism increased by 200 per cent, the number of pulsations while resting in bed 280, most acute tremor and exophthalmus, severest incompensation of the circulation. On my advice, Prof. Pommersheim implanted under the abdominal skin of the patient a piece of a struma rich in colloids which he resected from an otherwise healthy young girl. The result was highly remarkable. The patient, who only a few days before the operation collapsed while trying to leave his bed, was able to sit erect in his bed only two days after the operation and claimed not to have felt so well for years; on the 8th day he was out of bed, and on the 12th he was discharged with a normal number of pulsations and almost normal metabolism. He left for his home in a provincial town. Two months later he came back for a check-up of his condition, which proved completely satisfactory. Owing to the war further observation of this case was impossible. Although, thanks to the discovery of thiouracil, an operative treatment of Graves' disease may cease to be necessary, the described success of implanting thyroid tissue will retain its theoretical interest for the pathogenesis of this disease.

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